Development/Plasticity/Repair

Dosage-Dependent Effect of Dopamine D_2 Receptor Activation on Motor Cortex Plasticity in Humans

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The neuromodulator dopamine plays an important role in synaptic plasticity. The effects depend on receptor subtypes, affinity, concentration level, and the kind of neuroplasticity induced. In animal experiments, dopamine D_2-like receptor stimulation revealed partially antagonistic effects on plasticity, which might be explained by dosage dependency. In humans, D_2 receptor block abolishes plasticity, and the D_2/D_3, but predominantly D_3, receptor agonist ropinirol has a dosage-dependent nonlinear affect on plasticity. Here we aimed to determine the specific affect of D_2 receptor activation on neuroplasticity in humans, because physiological effects of D_2 and D_3 receptors might differ. Therefore, we combined application of the selective D_2 receptor agonist bromocriptine (2.5, 10, and 20 mg or placebo medication) with anodal and cathodal transcranial direct current stimulation (tDCS), which induces nonfocal plasticity, and with paired associative stimulation (PAS) generating a more focal kind of plasticity in the motor cortex of healthy humans. Plasticity was monitored by transcranial magnetic stimulation-induced motor-evoked potential amplitudes. For facilitatory tDCS, bromocriptine prevented plasticity induction independent from drug dosage. However, its application resulted in an inverted U-shaped dose–response curve on inhibitory tDCS, excitability-diminishing PAS, and to a minor degree on excitability-enhancing PAS. These data support the assumption that modulation of D_2-like receptor activity exerts a nonlinear dose-dependent effect on neuroplasticity in the human motor cortex that differs from predominantly D_3 receptor activation and that the kind of plasticity-induction procedure is relevant for its specific impact.

Key words: dopamine; dopamine receptors; neuroplasticity; paired associative stimulation; transcranial direct current stimulation; transcranial magnetic stimulation

Introduction

Dopamine modulates learning and memory formation. This effect is probably based on its affect on neuroplasticity, such as long-term potentiation (LTP) and long-term depression (LTD), as observed in animal studies (Jay, 2003). The precise mechanism of the dopaminergic affect on plasticity is complex and depends on receptor subtype, concentration level, and type of plasticity (Seamans and Yang, 2004; Kuo et al., 2008). Therefore, obtaining knowledge about dosage-dependent effects of specific dopamine receptor activation on synaptic plasticity *in vivo* in humans is critical.

Animal cognitive and human electrophysiological studies revealed nonlinear dosage-dependent effects of nonselective and D_1-like receptor activation on performance and plasticity (Seamans and Yang, 2004; Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011; Fresnoza et al., 2014). Insufficient or too much dopamine impairs performance, whereas an optimum

dose facilitates it (Williams and Goldman-Rakic, 1995). Accordingly, the affect of global dopaminergic and D_1 receptor activation on plasticity in humans differs with regard to dosage, plasticity-induction procedures, and direction of plasticity (facilitatory vs excitability diminution; Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011; Fresnoza et al., 2014). However, for the contribution of D_2-like receptors, variable effects on plasticity have been obtained in animal experimentation (Chen et al., 1996; Otani et al., 1998; Manahan-Vaughan and Kulla, 2003) and human cognitive studies (Breitenstein et al., 2006; Meintzschel and Ziemann, 2006). The D_2/D_3 agonist ropinirole revealed a nonlinear dosage-dependent effect on facilitatory, but not inhibitory, plasticity in humans (Monte-Silva et al., 2009). However, ropinirole predominantly activates D_3 rather than D_2 receptors (Coldwell et al., 1999). Animal experiments suggest different effects of D_2 and D_3 receptors on memory consolidation and locomotor activity in rats (facilitation by D_2 and inhibition by D_3 receptor activation; Kling-Petersen et al., 1995; Sigala et al., 1997). For neurotensin gene expression in rats, D_2 has a negative effect, whereas D_3 has a positive one (Diaz et al., 1994). For a full overview of the contribution of dopaminergic receptor subtypes on human brain plasticity, a clarification of the specific effect of D_2 receptor activation on plasticity in humans is warranted.

To this aim, we applied transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS) in combination with three doses (2.5, 10, and 20 mg and placebo) of bromocrip-

tine, a selective D_2 receptor agonist. tDCS induces a polarity-dependent, nonfocal type of glutamatergic plasticity (Nitsche et al., 2008). Anodal stimulation enhances excitability of the primary motor cortex, whereas cathodal tDCS diminishes it. PAS induces focal/synapse-specific glutamatergic plasticity of somatosensory–motor cortical connections. The mechanism resembles to a certain degree spike timing-dependent plasticity. The synchrony between motor cortex stimulation and an afferent somatosensory stimulus elicited by peripheral nerve stimulation determines the effect direction (Stefan et al., 2000, 2002; Wolters et al., 2003).

We hypothesized that specific D_2 receptor activation has a nonlinear affect on plasticity, which depends on the kind of plasticity-induction protocol.

Materials and Methods

Subjects. Twelve right-handed, healthy subjects participated in each experiment [tDCS experiment: seven males, five females, aged 27.92 ± 1.60 years (mean ± SD); and PAS experiment: seven males, five females, aged 28.42 ± 1.08 years (mean ± SD)]. Subjects with a history of medical diseases, metallic or electric implants in the body, intake of medication during or up to 2 weeks before participating in the study, and smokers and recreational drug users were excluded. Pregnancy was ruled out by a pregnancy test. Subjects gave written informed consent before participation. The study was approved by the Ethics Committee of the University of Göttingen and conforms to the Declaration of Helsinki.

Monitoring of corticospinal excitability. The peak-to-peak amplitudes of motor-evoked potentials (MEPs) induced over the motor cortex representation of the right abductor digiti minimi muscle (ADM) by transcranial magnetic stimulation (TMS) was used to monitor corticospinal excitability. Initially, single-pulse TMS generated by a Magstim 200 magnetic stimulator (Magstim) at a frequency of 0.25 Hz via a figure-of-eight magnetic coil (diameter of one winding, 70 mm; peak magnetic field, 2.2 tesla) was used to determine optimal coil position, defined as the site at which stimulation resulted in the largest MEP amplitudes. The coil was held tangentially to the scalp at an angle of 45° to the midsagittal plane with the handle pointing laterally and posteriorly, generating an anterior–posterior current direction in the brain. Electromyographic (EMG) recording was obtained from the right ADM with Ag–AgCl electrodes attached in a belly–tendon montage. Signals were filtered (30 Hz to 2 kHz), amplified (Digitimer 360; Digitimer), and then stored on a computer via a Power 1401 data acquisition interface (Cambridge Electronic Design). Analysis was performed using Signal Software (Cambridge Electronic Design). TMS intensity was adjusted to elicit baseline MEPs of averaged 1 mV peak-to-peak MEP amplitude and was kept constant for the post-stimulation assessment unless adjusted (see below).

Nonfocal plasticity induction by tDCS (Experiment 1). A battery-driven constant-current stimulator (NeuroConn) with a maximum output of 4.5 mA was used for tDCS via a pair of saline-soaked surface sponge electrodes each measuring 7 × 5 cm. We positioned one electrode over the motor cortex representation area of the right ADM and the other above the right supraorbital area. A current strength of 1 mA was administered for 13 min for anodal tDCS and 9 min for cathodal tDCS, which induces cortical excitability alterations lasting for ~1 h after the end of stimulation (Nitsche et al., 2008).

Focal plasticity induction by PAS (Experiment 2). A single TMS pulse with the stimulation intensity resulting in an MEP amplitude of ~1 mV was combined with a peripheral nerve stimulus (Digitimer D185 stimulator; Digitimer), which delivered an electrical pulse to the right ulnar nerve at the wrist level (cathode proximal, square waveform of 50 μs duration). The intensity was set to three times higher than the individual sensory perceptual threshold. Peripheral nerve stimulation was followed by the TMS stimulus with interstimulus intervals of 10 ms (inhibitory PAS: PAS10) for LTD-like plasticity induction or 25 ms (excitatory PAS: PAS25) for LTP-like plasticity induction. For PAS10, the somatosensory stimulus reaches the motor cortex relevantly earlier than the TMS stimulus applied over the motor cortex, whereas PAS25 results in synchro-

nous arrival of the somatosensory and TMS stimulus at this area. Ninety pairs of stimuli were administered at a frequency of 0.05 Hz for 30 min (Stefan et al., 2000, 2002; Wolters et al., 2003).

Pharmacological intervention. Two hours before the start of the plasticity-inducing protocols, the participants received low (2.5 mg), medium (10 mg), or high (20 mg) dosages of bromocriptine or placebo medication at each experimental session. These dosages cause systemic changes of cortical activity and performance (Kimberg et al., 2001; Franken et al., 2008). To prevent systemic side effects of bromocriptine, such as nausea and vomiting, subjects received 20 mg of the peripheral acting dopaminergic antagonist domperidone three times per day for 2 d before the experiment and also 2 h before bromocriptine intake. Domperidone at 20 mg alone exerts no effects on motor cortical excitability (Grundey et al., 2013).

Experimental procedures. The experiment was conducted in a double-blinded, randomized and placebo-controlled design. Each subject participated in eight sessions (Experiment 1 or 2) separated by an interval of at least 1 week to avoid interference effects. Subjects were seated on a reclining chair with head and arm support and were asked to relax but maintain their eyes open during the course of the experiment. EMG electrodes were placed at the right ADM using a belly–tendon montage. To ensure consistency, a skin marker was placed on the position of the EMG electrodes and motor cortex hotspot as identified by TMS. Then the TMS intensity that resulted in an MEP amplitude of ~1 mV was identified (SI1mV). At least 25 MEPs were recorded as baseline 1 with this stimulus intensity. Immediately after the baseline measurement, the participants received placebo medication or 2.5, 10, or 20 mg of bromocriptine. After 2 h, another set of 25 MEPs (baseline 2) was obtained to check for any drug-induced change of MEP amplitudes. If baseline 2 differed significantly (0.2 mV) from baseline 1, TMS intensity was readjusted to produce stable MEP amplitudes of ~1 mV (baseline 3). Then anodal tDCS (13 min), cathodal tDCS (9 min), PAS25, or PAS10 was applied. After intervention, 25 MEPs were recorded at the time points of 0, 5, 10, 15, 20, 25, 30, 60, 90, and 120 min, same day evening, next morning, next afternoon, and next evening (Fig. 1). Based on the results of previous studies, significant aftereffects at the post-intervention days could not be ruled out. This warrants the long-term monitoring of excitability in the present experiment (Kuo et al., 2008; Monte-Silva et al., 2009, 2010; Thirugnanasambandam et al., 2011).

Data analysis and statistics. The individual MEP amplitude means of baselines 1, 2, and 3 and all time points after plasticity induction were calculated. Post-intervention MEP amplitudes were normalized to baseline 2 only if baseline 2 did not differ significantly from baseline 1; otherwise, baseline 3 was used for normalization. Normalized MEP amplitudes were pooled together sessionwise by calculating the grand average across subjects for each condition and time point. After checking for normal distribution (Shapiro-Wilk test), a mixed linear model analysis (SPSS 21; SPSS) with subject as the random-effect covariate was applied with the MEP amplitude (as measured over time from baseline up to the next evening) as the dependent variable. Stimulation (tDCS and PAS), polarity (anodal and cathodal tDCS; PAS25 and PAS10), drug dosage (2.5, 10, and 20 mg of bromocriptine and placebo), time, and the respective interactions were treated as fixed-effect covariates. We used the partial η^2 calculated from an univariate ANOVA model to obtain effects sizes, because linear mixed models do not provide respective values. Fisher's least significant difference (LSD) *post hoc* tests (paired, two-tailed, $p < 0.05$), which do not correct for multiple comparisons, were performed to compare (1) the mean MEP amplitudes at all time points after tDCS or PAS versus baseline 2 or 3 and (2) the mean MEP amplitude obtained at a specific time point for the various drug conditions against the respective placebo medication condition. Baseline 1–3 MEP amplitudes were compared to test for any drug influence alone on cortical excitability and to exclude baseline differences between medication/stimulation conditions. Furthermore, we performed the same mixed linear model analysis with subjects as random factor for the standardized MEP amplitudes pooled for the first 60 min after plasticity induction. Then a Fisher's LSD *post hoc* test (paired, two-tailed, $p < 0.05$) was used to compare the first 60 min MEP amplitude of the respective placebo
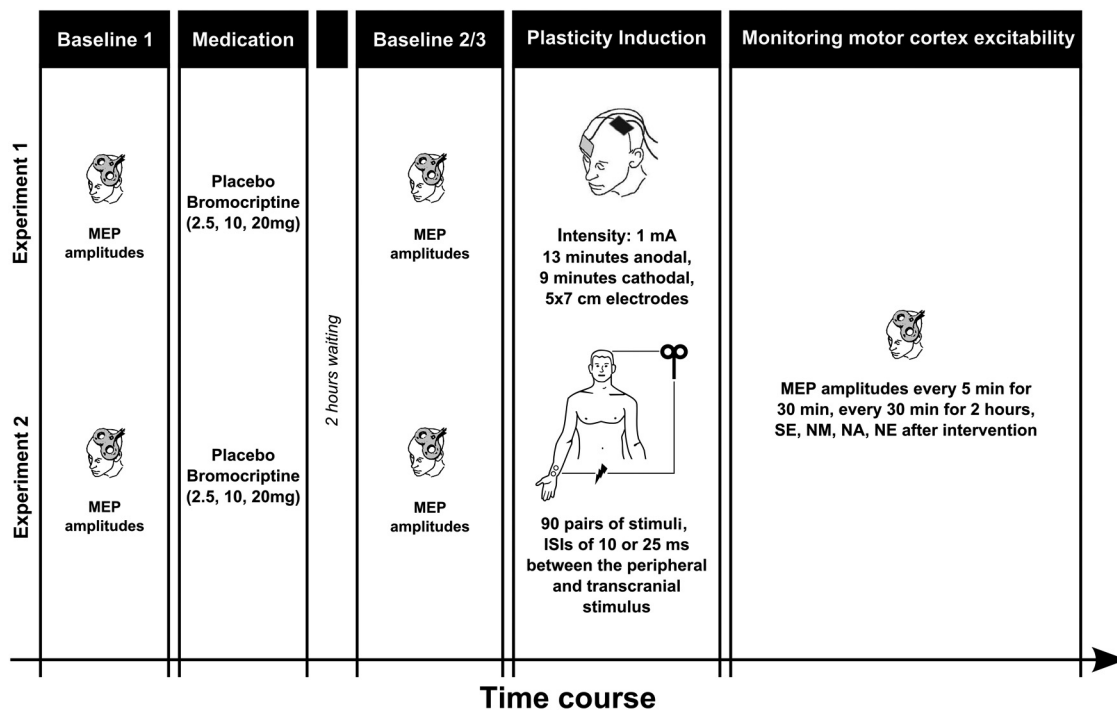
Figure 1. Course of the experiments. MEPs elicited by single-pulse TMS over the motor hotspot of the right ADM were recorded at 1 mV intensity before drug intake (baseline 1). Two hours after drug intake, baseline 2 was recorded to look for an effect of the drug on cortical excitability. In case of any MEP alterations from baseline 1, baseline 3 was recorded by adjusting the stimulator output to obtain a mean MEP amplitude of 1 mV. Then tDCS (anodal or cathodal) or PAS (excitatory or inhibitory) was administered, immediately followed by MEP after-measurements that covered 120 min. Additional after-measurements were performed at the same evening (SE) and the morning (NM), afternoon (NA), and evening (NE) of the second day after plasticity induction. ISI, Interstimulus interval.

Table 1. Peak-to-peak MEP amplitudes and TMS intensity before and after application of bromocriptine

Bromocriptine	Baseline 1		Baseline 2		Baseline 3	
	MEP (mV)	MSO (%)	MEP (mV)	MSO (%)	MEP (mV)	MSO (%)
Anodal tDCS						
2.5 mg	1.078 ± 0.03	41.5 ± 1.7	1.207 ± 0.15	41.5 ± 1.7	1.135 ± 0.03	40.4 ± 1.8
10 mg	1.122 ± 0.02	40.0 ± 1.5	1.067 ± 0.10	40.0 ± 1.5	1.064 ± 0.03	40.7 ± 1.6
20 mg	1.115 ± 0.02	41.5 ± 2.0	1.074 ± 0.06	41.5 ± 2.0	1.079 ± 0.02	39.0 ± 1.5
Cathodal tDCS						
2.5 mg	1.080 ± 0.03	42.2 ± 1.7	1.241 ± 0.12	42.2 ± 1.7	1.082 ± 0.03	39.7 ± 2.0
10 mg	1.051 ± 0.02	41.7 ± 2.1	1.021 ± 0.04	41.7 ± 2.1	1.040 ± 0.02	41.2 ± 0.7
20 mg	1.063 ± 0.02	42.2 ± 1.6	1.234 ± 0.20	42.2 ± 1.6	1.123 ± 0.02	43.0 ± 1.7
PAS25						
2.5 mg	1.071 ± 0.03	47.6 ± 2.8	0.971 ± 0.10	47.6 ± 2.8	1.045 ± 0.03	48.0 ± 3.2
10 mg	1.070 ± 0.02	47.6 ± 2.7	0.879 ± 0.13	47.6 ± 2.7	1.045 ± 0.02	46.4 ± 2.7
20 mg	1.106 ± 0.02	50.2 ± 3.5	0.966 ± 0.10	50.2 ± 3.5	1.120 ± 0.03	49.0 ± 2.1
PAS10						
2.5 mg	1.079 ± 0.03	50.2 ± 3.1	1.164 ± 0.14	50.2 ± 3.1	1.064 ± 0.03	49.8 ± 3.2
10 mg	1.119 ± 0.03	47.2 ± 2.7	0.966 ± 0.06	47.2 ± 2.7	1.041 ± 0.03	39.5 ± 3.0
20 mg	1.018 ± 0.02	47.7 ± 2.7	1.029 ± 0.10	42.3 ± 2.4	1.011 ± 0.03	50.4 ± 4.0

Shown are the mean MEP amplitudes and stimulation intensities [percentage of maximum stimulator output (MSO); mean ± SEM] of baselines 1–3. There was no significant difference between these parameters across the different conditions (Student's *t* test, paired, two-tailed, $p > 0.05$).

medication conditions with the first 60 min MEP amplitudes under real medication for all plasticity-induction protocols.

Results

With regard to side effects, 2 h after oral intake of 20 mg of bromocriptine, three subjects experienced dizziness, nausea, and vomiting, and hypotension was observed in one subject. One session had to be cancelled. Under low dosage, one subject experienced dizziness and one hypotension, and under medium dosage, only one subject developed dizziness. All symptoms were fully reversible, and the remaining subjects tolerated the drugs well.

Baseline peak-to-peak MEP amplitudes and baseline TMS intensity in percentage of maximal stimulator output were not affected by the drug and did not differ between conditions ($p \geq 0.05$, Student's paired, two-tailed *t* test; Table 1).

The data were normally distributed (Shapiro-Wilk test, all $p > 0.05$). Results of the mixed linear model analysis revealed significant effects of drug dosage (df = 3, $F = 20.015$, $p = < 0.001$, $\eta^2 = 0.020$), polarity (df = 1, $F = 139.812$, $p = < 0.001$, $\eta^2 = 0.046$), time course (df = 14, $F = 1.947$, $p = 0.015$, $\eta^2 = 0.010$), and significant interactions of stimulation × dosage (df = 3, $F = 4.337$, $p = 0.005$, $\eta^2 = 0.004$), dosage × polarity (df = 3, $F =$

Table 2. Results of the ANOVA conducted for tDCS and PAS

	df	F	p	η²
Stimulation	1	1.624	0.203	0.001
Dosage	3	20.015	<0.001*	0.020
Polarity	1	139.812	<0.001*	0.046
Time course	14	1.947	0.015*	0.010
Stimulation × dosage	3	4.337	0.005*	0.004
Stimulation × polarity	1	3.373	0.066	0.001
Dosage × polarity	3	75.237	<0.001*	0.073
Stimulation × dosage × polarity	3	5.111	0.002*	0.005
Stimulation × time course	14	0.756	0.718	0.004
Dosage × time course	42	0.882	0.687	0.013
Stimulation × dosage × time course	42	0.541	0.993	0.008
Polarity × time course	14	6.701	<0.001*	0.032
Stimulation × polarity × time course	14	0.379	0.981	0.002
Dosage × polarity × time course	42	3.361	<0.001*	0.047
Stimulation × dosage × polarity × time course	42	0.571	0.988	0.008

The ANOVA encompasses the time course of the MEP measures up to the next evening after stimulation. *$p <$ 0.05.

75.237, $p = <$ 0.001, $\eta^2 = 0.073$), stimulation × dosage × polarity (df = 3, $F = 5.111$, $p = 0.002$, $\eta^2 = 0.005$), polarity × time course (df = 14, $F = 6.701$, $p = <$ 0.001, $\eta^2 = 0.032$), and drug dosage × polarity × time course (df = 42, $F = 3.361$, $p = <$ 0.001, $\eta^2 = 0.047$; Table 2). Additional analysis of the MEP amplitudes for the first 60 min after stimulation using a mixed linear model with subjects as random factor revealed significant main effects of stimulation (df = 1, $F = 4.594$, $p = 0.032$), drug dosage (df = 3, $F = 18.018$, $p = <$ 0.001), polarity (df = 1, $F = 172.777$, $p = <$ 0.001), and significant interactions of stimulation × dosage (df = 3, $F = 3.070$, $p = 0.027$), polarity × dosage (df = 3, $F = 86.633$, $p = <$ 0.001), and stimulation × dosage × polarity interactions (df = 3, $F = 3.484$, $p = <$ 0.015).

Dose-dependent effect of D$_2$ receptor activation on tDCS-induced neuroplasticity

As revealed by the respective *post hoc* tests, under placebo medication, anodal tDCS increased excitability compared with baseline for up to 30 min after stimulation, whereas cathodal tDCS significantly decreased excitability for 25 min (Fig. 2A,B). Low-dosage bromocriptine prevented any effect of tDCS compared with the respective baseline MEP values. For anodal tDCS, the low-dosage bromocriptine condition consequently differed from placebo until the same evening, whereas for cathodal tDCS, the respective difference was significant for up to 25 min after stimulation (Fig. 2A,B). Under medium-dosage bromocriptine, the anodal tDCS-induced aftereffect was trendwise reversed until 30 min after stimulation and the evening of the second day. For cathodal tDCS, medium-dosage bromocriptine prolonged the MEP-reducing aftereffect significantly for up to 60 min after stimulation compared with baseline. Compared with placebo medication, the anodal tDCS-elicited aftereffect was significantly different from medium-dosage bromocriptine until 30 min after tDCS, whereas for cathodal tDCS, no significant difference between placebo and medium-dosage bromocriptine was observed (Fig. 2A,B). High-dosage medication resulted in similar effects as low-dosage medication on tDCS-generated excitability alterations. For anodal tDCS, MEP amplitudes did not differ from baseline values but were significantly reduced relative to placebo medication for up to 30 min after stimulation. For cathodal tDCS, similarly, MEP amplitudes did not differ relative to baseline but differed from cathodal tDCS-generated excitability reductions under placebo medication for 20 min (Fig. 2A,B). For the pooled MEP amplitudes (first 60 min after anodal tDCS), low

($p \leq 0.001$), medium ($p \leq 0.001$), and high ($p \leq 0.001$) dosage were significantly different compared with placebo medication (see Fig. 4A; *post hoc t* test, two-tailed, $p < 0.05$). However, only low ($p \leq 0.001$) and high ($p \leq 0.001$) dosage conditions were significant compared with placebo medication after cathodal tDCS (see Fig. 4A; (*post hoc t* test, two-tailed, $p < 0.05$). In summary, bromocriptine dosage dependently prevented or modified tDCS-induced excitability changes toward an excitability-diminishing direction. As can be seen from Figure 4B, relevant interindividual variability was present throughout the different medication conditions, especially with regard to low- and high-dosage bromocriptine application.

Dose-dependent effect of D$_2$ receptor activation on PAS-induced neuroplasticity

The results of the *post hoc* tests show that, under placebo medication, PAS25 increased excitability significantly compared with baseline for 30 min, whereas PAS10 decreased excitability for 60 min after stimulation compared with baseline (Fig. 3A,B). For low-dosage bromocriptine, excitatory and inhibitory PAS had no impact on MEP amplitudes compared with baseline. Compared with placebo medication, MEP amplitudes differed significantly until 90 min after PAS25 and PAS10 after plasticity induction (Fig. 3A,B). Under medium-dosage bromocriptine, MEP amplitudes were significantly enhanced versus baseline only for 20 min after excitatory PAS. In relation to the placebo medication condition, the respective excitability enhancement was significantly diminished for 30 min after plasticity induction. The MEP amplitudes after PAS10 were significantly reduced compared with baseline values until 90 min after stimulation. Compared with placebo medication, MEP amplitudes were not significantly different for PAS10 (Fig. 3A,B). High-dosage bromocriptine prevented any aftereffects of PAS compared with baseline MEP values. Consequently, MEP amplitudes differed significantly from those under placebo medication for 30 min after PAS25 and 25 min after PAS10 (Fig. 3A,B). For the pooled MEP amplitudes (up to 60 min after stimulation), the aftereffects of excitatory PAS under bromocriptine were significantly different compared with the placebo medication condition (low dose, $p \leq 0.001$); medium dose, $p \leq 0.001$; high dose, $p \leq 0.001$; Fig. 4A; *post hoc t* test, two-tailed, $p < 0.05$). For inhibitory PAS, low ($p \leq 0.001$) and high ($p \leq 0.001$) dosages of the drug were significantly different from the placebo medication condition, whereas the medium dosage ($p = 0.339$) did not result in significant differences (Fig. 4A; *post hoc t* test, two-tailed, $p < 0.05$). In summary, D$_2$ receptor activation by bromocriptine has a nonlinear dosage-dependent effect on PAS-induced plasticity: whereas low and high dosages prevented any PAS-induced neuroplasticity, the medium dosage preserved PAS10-induced LTD-like plasticity and did diminish, but not completely abolish, the aftereffects of PAS25. Similar to the tDCS data, considerable interindividual variability of the results can be seen in Figure 4B, which is largest for medium- and high-dosage bromocriptine for PAS25 and for the low and high dosage of the drug for PAS10.

Discussion

D$_2$ receptor activation had nonlinear dosage-dependent effects on motor cortex plasticity in humans. Low and high D$_2$ receptor activation prevented plasticity induction regardless of the specific stimulation protocol. Medium activation preserved inhibitory plasticity but diminished focal and prevented nonfocal facilitatory plasticity. These effects differ from those of combined D$_2$/D$_3$ activation (Monte-Silva et al., 2009).
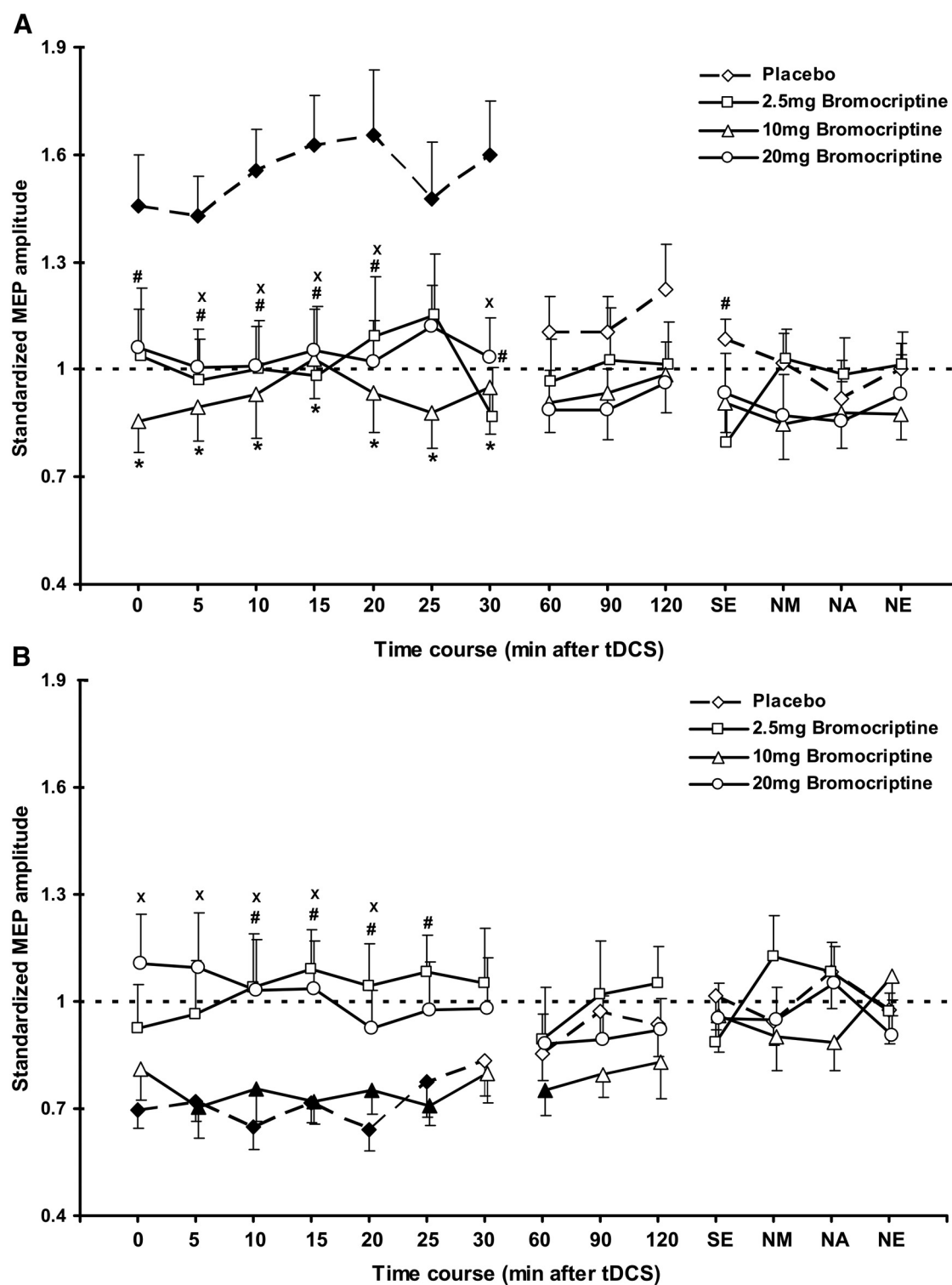
Figure 2. Dose-dependent effect of D$_2$ receptor activation on nonfocal plasticity induced by anodal and cathodal tDCS (Experiment 1). The *x*-axis displays the time points (in minutes) of after-measurements during the experiment. MEP amplitudes standardized to the corresponding baseline values (mean ± SEM) are plotted on the *y*-axis. The graphs show that, under placebo medication, anodal tDCS induces an excitability enhancement lasting for ∼30 min, whereas cathodal tDCS diminishes excitability for 25 min after stimulation. ***A***, Low-dose (2.5 mg), medium-dose (10 mg), and high-dose (20 mg) bromocriptine prevented the anodal tDCS-generated aftereffects. ***B***, Low-dose and high-dose bromocriptine prevented the cathodal tDCS-generated aftereffects, whereas under medium dose, the aftereffects were preserved. Filled symbols indicate statistically significant deviations of the post-tDCS MEP values compared with baseline. #, *, and × symbols indicate significant differences of the real medication compared with the placebo medication conditions at the same time points after plasticity induction (Fisher's LSD *post hoc* test, paired, two-tailed, *p* ≤ 0.05). SE, Same evening; NM, next morning; NA, next afternoon; NE, next evening. Error bars show SEM. #2.5 mg of bromocriptine, *10 mg of bromocriptine, and ×20 mg of bromocriptine.
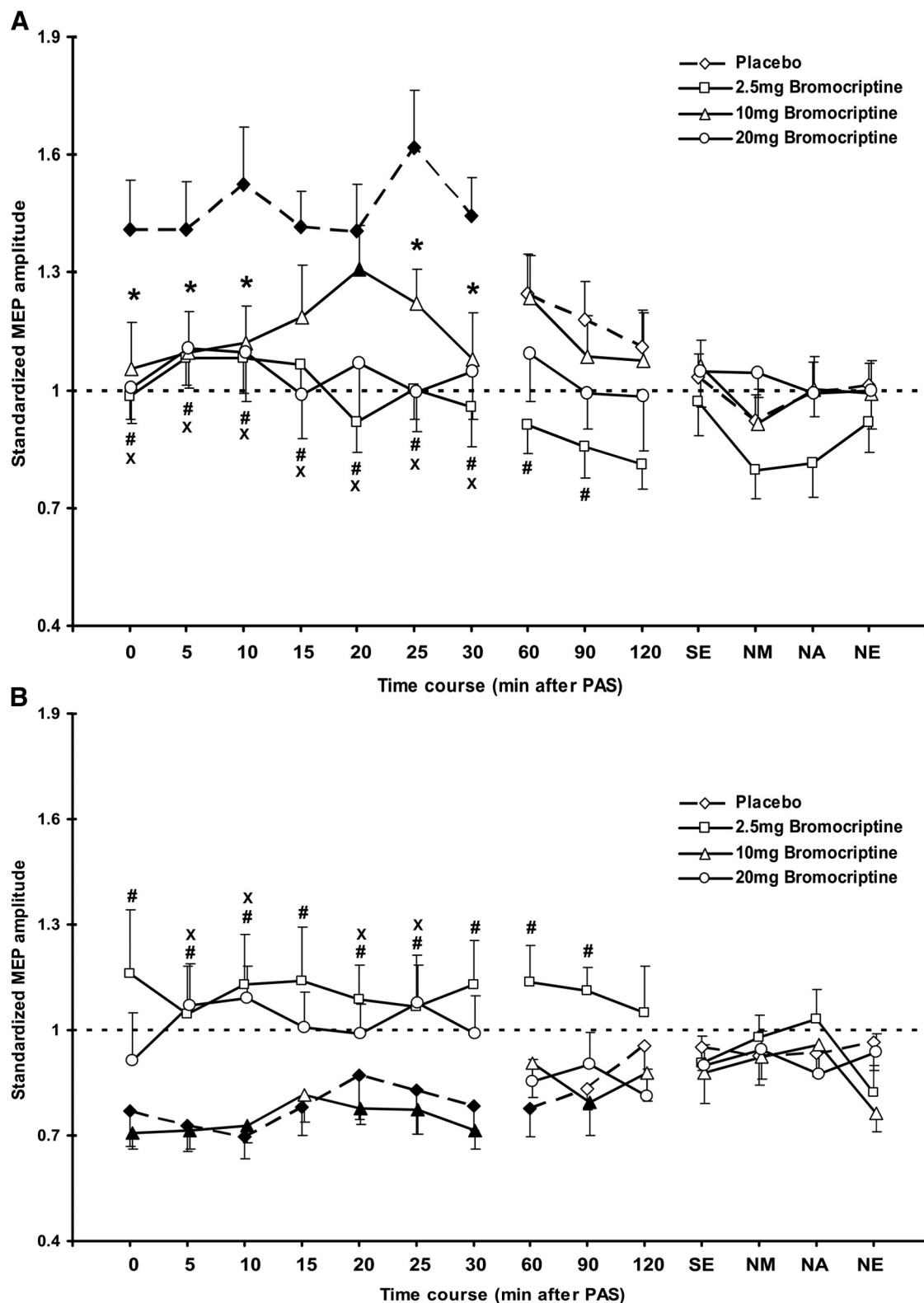
Figure 3. Dose-dependent effects of D_2 receptor activation on focal neuroplasticity induced by PAS25 and PAS10 (Experiment 2). The x-axis displays the time points (in minutes) of after-measurements during the experiment. MEP amplitudes standardized to the corresponding baseline values (mean $\pm$ SEM) are plotted on the y-axis. The graphs show that, under placebo medication, excitatory PAS (PAS25) induces an excitability enhancement lasting for ~30 min, whereas inhibitory PAS (PAS10) diminishes excitability for 60 min after stimulation. **A**, Low-dose (2.5 mg) and high-dose (20 mg) bromocriptine suppress the aftereffects of PAS25, whereas the medium dose (10 mg) reduced but did not abolish them. **B**, Low-dose (2.5 mg) and high-dose (20 mg) bromocriptine prevented the PAS10 aftereffects. Filled symbols indicate statistically significant deviations of the post-tDCS MEP values compared with baseline. #, *, and $\times$ symbols indicate significant differences of the real medication compared with the placebo medication conditions at the same time points after plasticity induction (Fisher's LSD *post hoc* test, paired, two-tailed, $p \leq 0.05$). SE, Same evening; NM, next morning; NA, next afternoon; NE, next evening. Error bars show SEM. #2.5 mg of bromocriptine, *10 mg of bromocriptine, and $\times$20 mg of bromocriptine.
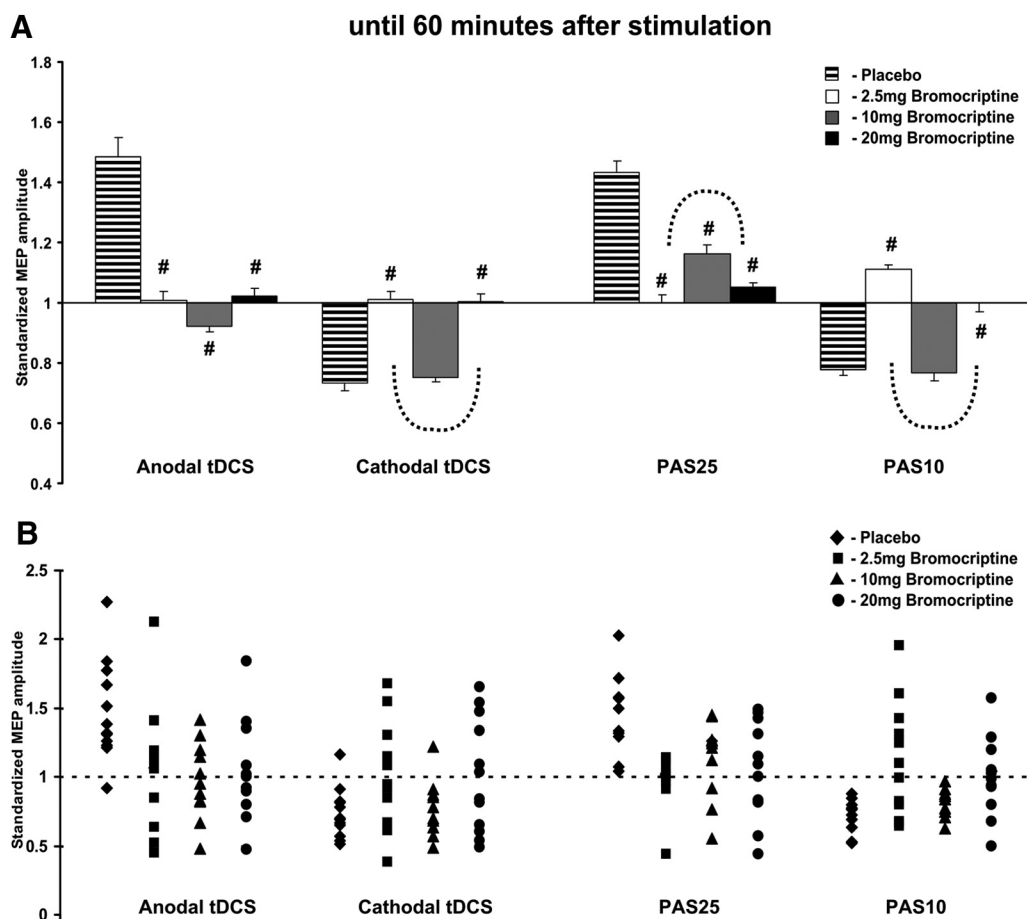
Figure 4. Dose-dependent effects of D$_2$ receptor activation on nonfocal and focal plasticity induced by tDCS and PAS. The horizontal line represents the baseline value of 1 mV before the start of the each stimulation condition, whereas the vertical line (*y*-axis) represent the MEP amplitudes standardized to the corresponding baseline values. *A*, D$_2$ receptor activation by bromocriptine has a nonlinear dosage-dependent effect on neuroplasticity induced by cathodal tDCS and inhibitory PAS. Low and high dosages impaired or prevented excitability alterations, whereas the medium dosage preserved these. In contrast, the effects of anodal tDCS and excitatory PAS were either impaired or prevented in all dosages. Each column represents the baseline-standardized MEP amplitudes pooled for 60 min after anodal/cathodal tDCS and PAS25/PAS10 from 24 participants. Error bars represent the SEM of the measurement immediately after until 60 min after stimulation. The # symbol indicates significant differences of the real medication compared with placebo medication (*post hoc t* test, paired, two-tailed, $p \leq 0.05$). *B*, Each point represents the mean of the MEP amplitude (calculated for the first 60 min after intervention) from each subject for each drug/stimulation condition combination. The results show considerable interindividual variability, especially under bromocriptine.

D$_2$ receptor modulation of LTP-like plasticity

D$_2$ receptor activation caused nonlinear dosage-dependent effects on LTP-like plasticity induced by focal plasticity induction and prevented nonfocal plasticity regardless of drug concentration. Low and high receptor activation prevented plasticity. Under medium-dosage bromocriptine, focal PAS-generated plasticity was diminished, whereas nonfocal facilitatory plasticity was prevented. Thus, D$_2$ activation, as performed in the present study, has a deleterious effect on LTP-like plasticity. This does not mean that D$_2$ receptor activity per se has a disruptive effect on LTP-like plasticity. D$_2$ receptor block abolished LTP-like plasticity in previous experiments (Nitsche et al., 2006, 2009), and in the present experiment, bromocriptine in any dosage enhanced D$_2$ activity on top of physiological activity. Therefore, physiological activity of D$_2$ receptors, which is compromised by both D$_2$ receptor block and hyperactivity induced by bromocriptine, might be necessary for optimal plasticity induction. However, because bromocriptine had heterogeneous effects on cognitive functions in humans (Luciana et al., 1992; Kimberg et al., 1997; Luciana and Collins, 1997; Müller et al., 1998; Mehta et al., 2001) including improvement, it might also be argued that state-dependent heterogeneous optimal physiological levels of D$_2$ activity do exist,

which to a certain degree are mimicked by pharmacological intervention. In accordance, global activation of the dopaminergic system and predominant activation of D$_3$ or D$_1$ receptors did not in each case disrupt plasticity (Monte-Silva et al., 2009, 2010; Thirugnanasambandam et al., 2011; Fresnoza et al., 2014). The nonlinear effect of D$_2$ receptor activation on focal LTP-like plasticity is in accordance with results of D$_2$/D$_3$ receptor activation and of nonselective dopamine receptor activation (Monte-Silva et al., 2009; Thirugnanasambandam et al., 2011). However, the plasticity-preventing effect of bromocriptine on nonfocal plasticity (anodal tDCS) under the medium dose differs from the conversion to LTD-like plasticity accomplished via global dopamine receptor activation (Kuo et al., 2008; Monte-Silva et al., 2010), suggesting a role of D$_1$ receptor activation for this type of plasticity (Fresnoza et al., 2014). It also differs from the results obtained by predominantly D$_3$ receptor activation, which preserved the respective aftereffects. The differences between D$_2$ and D$_3$ receptor activation might be mechanistically explained by the fact that D$_3$ receptors modulate cortical activity by coactivation with D$_1$ receptors (Avalos-Fuentes et al., 2013). Thus, enhanced activation of D$_3$ receptors together with spontaneous D$_1$ activity could

cause the effects of ropinirole. Accordingly, medium-dosage D$_1$ activation resulted in similar effects (Nitsche et al., 2009).

The effect of D$_2$ receptor activation on LTP-like plasticity can be explained by presynaptic and postsynaptic effects. Low concentrations of the drug act primarily on presynaptic autoreceptors, thus reducing freely available dopamine (Benoit-Marand et al., 2001), which would result in reduced glutamatergic activity and calcium release needed for plasticity induction (Lisman, 2001). Indeed, reduction of postsynaptic dopaminergic activity has been shown to prevent tDCS- and PAS-induced plasticity (Nitsche et al., 2006, 2009). For the medium dosage, D$_2$ receptor stimulation diminished the excitatory effect of PAS25 and prevented plasticity induction by anodal tDCS. This can be explained by the activation of postsynaptic D$_2$ receptors, which diminish GABAergic and glutamatergic receptor activity (Seamans and Yang, 2004). For PAS, which induces phasic suprathreshold synaptic activation, the reduction of glutamate-driven calcium influx in the postsynaptic neuron might have not been sufficient to block LTP-like plasticity but sufficient to reduce the tonic, lower-level calcium influx induced by subthreshold tDCS to prevent plasticity. Alternatively, GABA reduction might have caused a lack of inhibition and thus might have resulted in calcium overflow predominantly for tDCS-induced LTP-like plasticity, which could result in larger calcium levels compared with PAS, because it is synaptically less restricted and causes tonic calcium influx. In accordance, excessive duration of anodal tDCS induces LTD-like plasticity (Monte-Silva et al., 2013). These mechanisms would then be responsible for the abolishment of LTP-like plasticity independent from the induction procedure in case of high-level D$_2$ receptor activation.

D$_2$ receptor modulation of LTD-like plasticity

The affect of D$_2$ receptor activation on LTD-like plasticity follows an inverted U-shaped curve, strengthening the assumption that LTD depends on D$_2$ receptor activation (Wilson, 2006) and consistent with the effect of global dopaminergic activation on cathodal tDCS and PAS10 (Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011), as well as with the impact of D$_3$/D$_2$ receptor activation on cathodal tDCS (Monte-Silva et al., 2009). Conversely, ropinirole had no affect on PAS10-induced plasticity at any dosage.

Mechanisms of action might be similar to the effect of D$_2$ agonism on LTP-like plasticity, because PAS10 and cathodal tDCS also induce glutamatergic and calcium-dependent plasticity (Liebetanz et al., 2002; Wolters et al., 2003). Under low-dosage bromocriptine, D$_2$ autoreceptor activation could prevent plasticity attributable to reduced dopamine release. For the medium dose, the preservation of both focal and nonfocal LTD-like plasticity would have been caused by a sufficient calcium influx via D$_2$ activation for LTD-like plasticity induction. High-dosage bromocriptine would abolish the aftereffects of both cathodal tDCS and inhibitory PAS by exceeding calcium increase caused by reduced GABAergic inhibition. In accordance, cathodal tDCS with an intensity of 2 mA, which should result in larger calcium increase compared with 1 mA stimulation as applied in the present study, induces no LTD-like plasticity (Batsikadze et al., 2013). Alternatively, D$_2$ receptor-dependent reduction of NMDA receptor activation (Seamans and Yang, 2004) could have abolished plasticity because both cathodal tDCS and PAS10 require NMDA receptor activation.

General remarks

The present study together with previous experiments (Nitsche et al., 2006, 2009; Kuo et al., 2008; Monte-Silva et al., 2009, 2010) adds information about the contribution of dopamine receptor subtypes to neuroplasticity. Our results support to some extent the contribution of D$_2$ receptors to the "focusing effect" (strengthening of focally induced but weakening/conversion of nonfocally induced LTP-like plasticity) observed under global dopaminergic activation (Kuo et al., 2008). Focal facilitatory plasticity was diminished but not abolished, whereas non-focal plasticity was prevented. This effect might underlie the signal-to-noise modulation that dopamine exerts on task-relevant neural processes. Accordingly, optimal dopamine levels would modulate task-related neural processing, allowing for flexible use of information, whereas a low level of dopamine would reduce the likelihood that a memory trace will be retained and excessive dopamine levels prevent the updating or replacement of information in current memory stores (Durstewitz et al., 2000). Although these mechanisms were proposed primarily for working memory functions, the results of our studies propose that they might also be relevant for long-term memory storage. The nonlinear effects of D$_2$ receptor stimulation on plasticity do not only imply the need for an optimal degree of D$_2$ receptor activation but might also explain the contribution of D$_2$ receptor overactivity for the development of psychotic symptoms (Seeman and Kapur, 2000). Specifically, the lack of plasticity present under high activation of D$_2$ receptors might enhance noise in brain networks and therefore lead to erroneous information processing. Furthermore, the involvement of D$_2$-like receptors in the facilitatory control of memory consolidation (Sigala et al., 1997) might have a promising therapeutic potential for patients suffering from Parkinson's disease, in which rehabilitation involves improving cognitive functions as well.

Some potential limitations of the present study should be taken into account. First, the mechanistic explanation of the results is speculative at present. However, our findings correlate well with known D$_2$ receptor action. Second, high variability in the response to tDCS was reported recently (Wiethoff et al., 2014), and variability, although to a somewhat minor degree, was also present in this study. Differences of variability between studies might be caused by the fact that our plasticity-induction protocols differ from that performed in the aforementioned study. Stimulation with an intensity of 2 mA, compared with 1 mA stimulation as performed in the present study, might result in nonlinear effects of tDCS (Batsikadze et al. 2013). Third, dopaminergic medication seems to enhance interindividual variability. This is probably caused by the dosage-dependent modulatory effect of dopamine, which could in combination with genetic polymorphisms (Wong et al., 2000; Witte and Flöel, 2012; Witte et al., 2012; Kristin et al., 2013) and differences of resorption of the substance, which we both did not explore, result in interindividually different activation of D$_2$ receptors despite identical dosages. Finally, blinding might have been compromised in one subject for whom the respective session had to be cancelled as a result of vomiting after the high dosage of bromocriptine. However, in the majority of subjects, side effects, if present, occurred across different dosages of the drug; thus, blinding should have been maintained in these cases. Additionally, multiple sessions, blinded tDCS and PAS protocols, medication, and identical aftermeasurement durations in all conditions should have guaranteed blinding in general.

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