

D₁-Receptor Impact on Neuroplasticity in Humans

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Dopamine improves learning and memory formation. The neurophysiological basis for these effects might be a focusing effect of dopamine on neuroplasticity: Accordingly, in humans L-dopa prolongs focal facilitatory plasticity, but turns nonfocal facilitatory plasticity into inhibition. Here we explore the impact of D₁ receptors on plasticity. Nonfocal plasticity was induced by transcranial direct current stimulation (tDCS), and focal plasticity by paired associative stimulation (PAS). Subjects received sulpiride, a D₂ antagonist, to increase the relative contribution of D₁ receptors to dopaminergic activity, combined sulpiride and L-dopa, to increase the relation of D₁/D₂ activity further, or placebo medication. Under placebo, anodal tDCS and excitatory PAS (ePAS) increased motor cortex excitability. Cathodal tDCS and inhibitory PAS (iPAS) reduced it. Sulpiride abolished iPAS-induced inhibition, but not ePAS-generated facilitation, underlining the importance of D₁-receptor activity for focal facilitatory neuroplasticity. Combining sulpiride with L-dopa reestablished iPAS-induced inhibition, but did not affect ePAS-induced plasticity. tDCS-induced plasticity, which was abolished by sulpiride in a former study, also recovered. Thus enhancing D₁ activity further relative to D₂ activity is relevant for facilitatory and inhibitory plasticity. However, comparison with former results show that an appropriate balance of D₁ and D₂ activity seems necessary to (1) consolidate the respective excitability modifications and (2) to elicit a focusing effect.

Key words: dopamine; human; motor cortex; neuroplasticity; paired associative stimulation; transcranial direct current stimulation

Introduction

Dopamine (DA) modulates learning and memory. Dopaminergic enhancement improves verbal learning in healthy subjects and motor learning in healthy subjects and stroke patients (Knecht et al., 2004; Flöel et al., 2005a; Floel et al., 2005b). Cognitive functions are impaired in diseases accompanied by dopaminergic dysfunction, like Parkinson's disease and schizophrenia (Grace et al., 1998).

The neurophysiological basis for these effects of DA, as primarily studied in animal and slice experiments, might be its prolonging effect on neuroplasticity, i.e., long-term potentiation (LTP) and depression (LTD) (Otani et al., 1998; Bailey et al., 2000). D₁-receptor activation prolongs LTP and LTD (Chen et al., 1996; Otmakhova and Lisman, 1998; Bach et al., 1999; Bailey et al., 2000; Gurden et al., 2000; Huang et al., 2004). For D₂ receptors, positive as well as negative effects on LTP and LTD are described (Frey et al., 1989; Chen et al., 1996; Otani et al., 1998; Gurden et al., 2000; Spencer and Murphy, 2000; Manahan-Vaughan and Kulla, 2003). Thus DA has no uniform excitatory or inhibitory function, but acts as neuromodulator. On cortical network level, this results in a focusing effect of DA on cortical

activity and plasticity, i.e., an enhancement of the signal-to-noise ratio (Foote and Morrison, 1987; Seamans and Yang, 2004).

This focusing effect was confirmed in humans by use of neuroplasticity-inducing stimulation tools, namely transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS), which elicit LTP- and LTD-like effects on cortical excitability in terms of time course [excitability changes lasting for ~1 h (Stefan et al., 2000; Nitsche and Paulus, 2001; Nitsche et al., 2003a; Wolters et al., 2003), and NMDA receptor dependence (Stefan et al., 2002; Nitsche et al., 2003b)]. PAS induces focal plasticity by modification of the strength of somatosensory-motor cortical connections (Stefan et al., 2000). tDCS induces nonfocal plasticity within the motor cortex not restricted to synaptic subgroups (Purpura and McMurtry, 1965; Nitsche et al., 2003c, 2008). Since L-dopa prolongs the focal excitability enhancement induced by excitatory PAS (ePAS), turns anodal tDCS-generated nonfocal excitability enhancement into inhibition and prolongs cathodal tDCS-induced excitability diminution (Kuo et al., 2008), it has a focusing effect on excitatory plasticity. With regard to dopaminergic subreceptors, D₂-antagonism eliminates tDCS-induced plasticity, whereas D₂-agonism prolongs the inhibitory effect of cathodal tDCS (Nitsche et al., 2006). Thus D₂-receptor activity is essential for stabilizing nonfocal inhibitory plasticity and might also contribute to nonfocal excitatory plasticity.

We aimed to shed light on the relevance of D₁-receptor activation for plasticity in humans. Since no selective D₁ agonist or antagonist is available for use in humans, we chose an indirect approach. In the first experiment, the balance of D₁/D₂ receptor activation was shifted toward the D₁ receptor by blocking D₂ receptors with sulpiride. We explored the impact of this drug solely on focal, PAS-induced plasticity, because its effect on non-

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focal, tDCS-induced plasticity was already tested (Nitsche et al., 2006). In the second experiment, D₁ activation was enhanced by combining L-dopa with sulpiride. The effects of this drug combination on plasticity were explored for PAS- and tDCS-induced plasticity. The primary motor cortex was taken as a model system.

Materials and Methods

Subjects. Ten to 12 healthy subjects without acute or chronic CNS-affecting medication participated in each experiment (experiment 1 (sulpiride/placebo) 10 subjects, 5 female, mean age 27.1 years \pm 6.10 SD; experiment 2 (sulpiride-L-dopa/placebo) 12 subjects, 7 female, mean age 30.67 \pm 10.53 SD). All gave written informed consent. The study was approved by the ethics committee of the University of Göttingen, and we conform to the Declaration of Helsinki.

tDCS of the motor cortex. tDCS was applied to induce nonfocal neuroplasticity. It generates a modulation of cortical network plasticity by application of weak direct currents through the surface of the scalp. Depending on stimulation duration anodal tDCS enhances and cathodal tDCS diminishes cortical excitability for about an hour after the end of stimulation (Nitsche and Paulus, 2001; Nitsche et al., 2003a). The primary mechanism is a modulation of resting membrane potential, and the resulting polarity-specific excitability and changes in cortical activity subsequently induce changes in synaptic strength; which are however not restricted to specific synaptic subgroups, since excitability and activity of a broad range of cortical neurons is modulated by tDCS, as shown in animal experiments (Purpura and McMurtry, 1965; Nitsche et al., 2003b). The after-effects of tDCS are NMDA receptor-dependent (Nitsche et al., 2003c).

Direct currents were transferred via a pair of saline-soaked surface sponge electrodes (35 cm²) fixed to the scalp and delivered by a specially developed battery-driven constant current stimulator (Schneider Electronic) with a maximum output of 2 mA. The motor-cortical electrode was placed over the representational field of the right abductor digiti minimi (ADM) as identified by transcranial magnetic stimulation (TMS), and the other electrode was located contralaterally above the right orbit. The currents flowed continuously for 9 (cathodal tDCS) and 13 (anodal tDCS) min with an intensity of 1.0 mA. These stimulation durations have been shown to elicit the intended excitability shift durations in former experiments (Nitsche and Paulus, 2001; Nitsche et al., 2003a).

PAS of the motor cortex. For PAS, repetitive peripheral nerve stimulation is paired with TMS of the human motor cortex (Stefan et al., 2000). It is postulated that PAS-induced excitability changes specifically facilitate somatosensory-motor cortical connections. Recently, it has moreover been demonstrated that the effects of PAS in the human motor cortex are restricted to the motor cortical representations affected by the stimulation protocol, but do not spread to neighbored ones (Weise et al., 2006). The efficacy of PAS to induce motor cortical excitability alterations specifically depend on the interstimulus interval. Thus, PAS shares some critical features of associative synaptic LTP and LTD (Stefan et al., 2000, 2002; Wolters et al., 2003). PAS-induced excitability changes have been demonstrated to be NMDA receptor-dependent (Stefan et al., 2002).

Peripheral nerve stimulation was applied to the right ulnar nerve at the level of the wrist. Single-pulse TMS was delivered over the representing area of the right ADM and preceded by an ulnar nerve stimulus with an interval of 25 ms (ePAS) or 10 ms [inhibitory PAS (iPAS)] with stimulation intensity of 300% of the perceptual threshold. With an interstimulus interval of 25 ms, the somatosensory afferent stimulus elicited by peripheral stimulation reaches the motor cortex nearly simultaneously with the TMS stimulus delivered directly to the motor cortex. The resulting simultaneous activation of a postsynaptic neuron delivered by conjoint afferent inputs has been shown to induce LTP in animals (Baranyi et al., 1991; Hess and Donoghue, 1994). In the case of the 10 ms interstimulus interval, however, the somatosensory afferent stimulus reaches the respective motor cortical neurons relevantly later than the TMS stimulus. This a-synchronous activation has been shown to induce LTD in animals (Zhang et al., 1998). Both effects might be dependent on the resulting

calcium concentration of the postsynaptic neurons. Simultaneous activation might induce a strong enhancement of intraneuronal calcium sufficient for the induction of LTP, while asynchronous activation might enhance calcium to a lower degree, thus resulting in LTD (Lisman, 2001). Ninety pairs were applied at 0.05 Hz >30 min, which has been shown to induce a long-lasting excitability modification of the motor cortex (Stefan et al., 2000).

Pharmacological interventions. Four-hundred milligrams of sulpiride, a combination of 400 mg of sulpiride and 100 mg of L-dopa (combined with 20 mg of domperidone to avoid side-effects, e.g., vomiting, of the medication) or equivalent placebo (PLC) drugs were taken by the subjects 1 h before the start of the experimental session. By this means, the *verum* drugs induce stable plasma levels and produce prominent effects in the CNS (Flöel et al., 2005a; Nitsche et al., 2006; Kuo et al., 2008). Each experimental session was performed in a randomized order and was separated by at least 1 week to avoid cumulative drug effects. Subjects were blinded to the drugs received in the specific sessions.

Measurement of motor cortical excitability. TMS-elicited muscle-evoked potentials (MEPs) were recorded to measure excitability changes of the representational motor cortical area of the right ADM. Single-pulse TMS was conducted by a Magstim 200 magnetic stimulator (Magstim Company) with a figure-of-eight magnetic coil (diameter of one winding = 70 mm, peak magnetic field = 2.2 Tesla). The coil was held tangentially to the skull, with the handle pointing backwards and laterally at an angle of 45° from midline. The optimal position was defined as the site where stimulation resulted consistently in the largest MEPs. Surface EMG was recorded from the right ADM with Ag-AgCl electrodes in a belly-tendon montage. The signals were amplified and filtered with a time constant of 10 ms and a low-pass filter of 2.5 kHz, then digitized at an analog-to-digital rate of 5 kHz and further relayed into a laboratory computer using the Signal software and CED 1401 hardware (Cambridge Electronic Design). The intensity was adjusted to elicit baseline MEPs of, on average, 1 mV peak-to-peak amplitude and was kept constant for the poststimulation assessment unless adjusted (see below).

Experimental procedures. The experiments were conducted in a repeated measurement design. First the optimal position of the magnetic coil for eliciting MEPs in the resting ADM was assessed over the left motor cortex and 20 MEPs were recorded for the first baseline. One hour after intake of the medication, a second baseline was determined to control for a possible influence of the drug on cortical excitability and adjusted if necessary. Subjects were not aware about and could not distinguish between the specific stimulation protocols used nor were they informed about the medication administered in a specific session.

In the experiments with tDCS, one of the DC electrodes, to which in the following the terms cathodal or anodal tDCS refer, was fixed at the cortical representational area of ADM as defined during the first baseline recording, and the other one was fixed at the contralateral forehead area above the right orbit. Direct currents were applied on 12 subjects for 9 (cathodal) or 13 min (anodal). After cessation of tDCS, 20 MEPs were recorded at 0.25 Hz every 5 min for half an hour, and then every 30 min until 2 h after the end of DC stimulation, since tDCS-induced after-effects without medication will not last longer than this period of time (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2003a). Because of the relatively diurnal stability of corticospinal excitability, we did not expect further excitability changes after MEPs returned to baseline level again. For the medication conditions, TMS recordings were performed also at the evening of the stimulation day [same day evening (SE)], and the day after plasticity-inducing intervention [next morning (nm), next noon (nn), and next evening (ne)] in experiment 2.

In the experiments with PAS, the interventional PAS protocols as described above were used. TMS recording procedures were the same as described above. Each experimental session was performed in a randomized order and was separated by at least 1 week to avoid cumulative drug or stimulation effects.

A synopsis of the experimental procedures is shown in Figure 1. Experiment 1, which tested the impact of sulpiride only on plasticity, was only conducted for PAS, and not for tDCS, because the latter experiment was already performed in a former study of our group (Nitsche et al.,

2006). Experiment 2, i.e., the sulpiride/L-dopa drug combination, was performed for tDCS (experiment 2a), and PAS (experiment 2b).

Data analysis and statistics. MEP amplitude means were calculated first individually, then interindividually for each time bin including the baseline values (before medication, 1 h after medication, TMS intensity not adjusted and 1 h after medication, TMS intensity adjusted), to detect medication-induced baseline modifications. The postintervention MEPs were normalized and are given as ratios of the third baseline.

For the tDCS and PAS experiments, repeated measure ANOVAs for the time bins up to 120 min after stimulation were calculated with the within-subject factors time course, current stimulation (anodal and cathodal tDCS, ePAS, and iPAS), drug condition [sulpiride (experiment 1), sulpiride/L-dopa (experiment 2) vs placebo], and the dependent variable MEP amplitude. If appropriate, *post hoc* Student's *t* tests (paired samples, two-tailed, $p < 0.05$, not adjusted) were performed to determine whether the MEP amplitudes before and after the interventional brain stimulations differed in each intervention condition, and if those differences depended on the drug conditions. Additional *post hoc* tests (Student's *t* tests, $p < 0.05$) were performed to explore whether sulpiride or the combination of sulpiride and L-dopa modified baseline MEPs.

Results

Beyond a slight itching sensation under the tDCS electrodes and a discrete dizziness under medication in 3 subjects under the sulpiride/L-dopa combination, no side-effects of stimulation or pharmacological intervention were reported by the subjects.

Effect of sulpiride on focal plasticity induced by PAS (experiment 1)

The ANOVA shows significant main effects of time and PAS. Moreover, the interactions time \times drug and time \times PAS were significant (Table 1). The excitability enhancement accomplished by ePAS was significant up to 30 min after stimulation independent of the drug condition. During the first minutes after stimulation, a nonsignificant trend for an enlarged excitability enhancement under sulpiride, compared with PLC medication, can be noticed. For iPAS, however, a significant reduction of the MEP amplitude was apparent for 25 min under placebo medication only. Sulpiride abolished this effect of iPAS (Fig. 2).

Effect of L-dopa combined with sulpiride on tDCS-induced plasticity (experiment 2a)

The ANOVA resulted in a significant main effect of tDCS and in a significant interaction of tDCS \times time (Table 1). The excitability enhancement accomplished by anodal tDCS was stable for ~ 30 min after the end of stimulation, independent from real or placebo medication. However, a nonsignificant trend for an enlarged excitability enhancement under sulpiride and L-dopa, compared with PLC medication, can be noticed. In the cathodal tDCS condition, under placebo medication MEP amplitudes were significantly reduced for up to

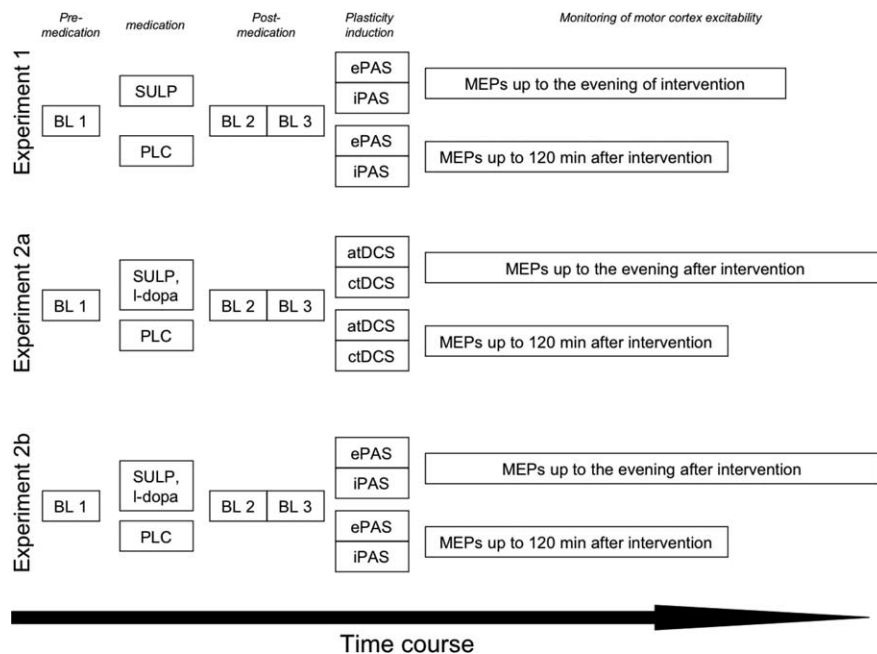


Figure 1. Synopsis of experimental procedures. For each experiment, before drug intake a first baseline MEP measure (BL1) was performed (determination of TMS intensity resulting in an MEP amplitude of 1 mV). Immediately afterward, drug intake followed. In experiment 1, the subjects received sulpiride (SULP) or PLC medication, in experiment 2, they received a combination of SULP and L-dopa or PLC. One hour after drug intake, a second baseline (BL2) was recorded with the TMS intensity of BL1. If MEP amplitudes were different from the first baseline, TMS intensity was adjusted such that a MEP amplitude of 1 mV (BL3) resulted. The TMS intensity of BL3 was kept constant during the remaining experiment. Afterward, the plasticity induction procedures (only PAS in experiment 1, PAS and tDCS in experiment 2) were performed. Post-interventionally MEPs were recorded for 30 min every fifth minute, then every 30th min up to 120 min for each condition. For PLC medication, MEP recording was terminated afterward, because it is known that baseline excitability is reached after approximately 1 h after tDCS and PAS without pharmacological intervention. For the drug conditions, MEPs were recorded once more at the evening of the day under SULP and until the evening of the following day under SULP/L-dopa to cover also prolonged excitability changes. atDCS, Anodal tDCS; ctDCS, cathodal tDCS.

90 min after tDCS, while for only 30 min after tDCS under L-dopa and sulpiride (Fig. 3a).

Effect of L-dopa combined with sulpiride on PAS-induced plasticity (experiment 2b)

Here the ANOVA resulted in significant main effects of PAS and time. The interaction PAS \times time was also significant (Table 1). Medication status had no significant influence on the excitability diminution or enhancement accomplished, which was prominent for ~ 30 min in both, the drug and placebo condition. Again a nonsignificant trend for an enlarged excitability enhancement under sulpiride and L-dopa, compared with PLC medication, can be noticed. (Fig. 3b).

Drug-induced baseline MEP modifications

In experiment 1, sulpiride alone did not modify corticospinal excitability. MEP amplitudes were identical before (1.022 ± 0.092 SD) and 1 h after medication (1.042 ± 0.204 SD, $t = 0.353$, $p = 0.730$). With regard to experiment 2, however, the combination of L-dopa and sulpiride diminished MEP amplitudes slightly, but significantly [1.010 ± 0.084 SD before medication (baseline 1), 0.811 ± 0.219 after medication (baseline 2), $t = 4.5$, $p < 0.001$]. After adjustment, baseline MEP values were identical for each plasticity-inducing protocol immediately before the plasticity-inducing procedure (baseline 3).

Discussion

The results of our study show a nonlinear contribution of D_1 receptor activity to focal and nonfocal plasticity in the human

Table 1. Results of the ANOVAs conducted for experiment 1 (sulpiride) and experiment 2 (sulpiride and L-dopa)

Experiment	df	F	p
Sulpiride or placebo medication, PAS			
Time	10	6.625	<0.001*
Drug	1	4.763	0.057
PAS	1	33.172	<0.001*
Time × drug	10	3.075	0.002*
Time × PAS	10	6.434	<0.001*
Drug × PAS	1	1.984	0.193
Time × drug × PAS	10	0.875	0.559
Sulpiride/L-dopa or placebo medication, tDCS			
Time	10	1.568	0.126
Drug	1	1.034	0.331
tDCS	1	58.192	<0.001*
Time × drug	10	0.831	0.600
Time × tDCS	10	13.323	<0.001*
Drug × tDCS	1	0.414	0.533
Time × drug × tDCS	10	0.931	0.508
Sulpiride/L-dopa or placebo medication, PAS			
Time	10	2.428	0.012*
Drug	1	2.089	0.176
PAS	1	21.658	0.001*
Time × drug	10	1.860	0.059
Time × PAS	10	11.223	<0.001*
Drug × PAS	1	0.016	0.903
Time × drug × PAS	10	1.251	0.267

Note that for both experiments, the ANOVA encompasses the time course up to 120 min after tDCS, because the remaining time points were only measured for the medication conditions. Asterisks indicate significant results ($p < 0.05$).

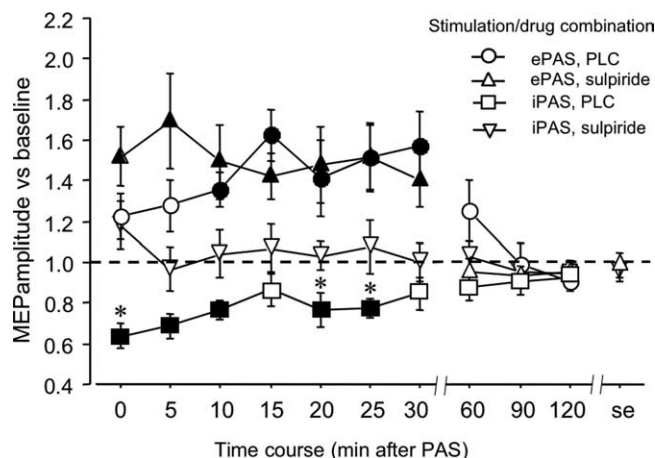


Figure 2. Impact of D₂ block on focal plasticity induced by ePAS and iPAS (experiment 1). Depicted are the baseline-standardized MEP amplitudes up to the evening after ePAS or iPAS under placebo medication or sulpiride. Under placebo medication, ePAS enhances while iPAS diminishes excitability significantly for up to 30 min after stimulation. Under sulpiride, the ePAS-generated excitability enhancement remains largely unaffected, whereas sulpiride abolishes the iPAS-induced excitability reduction. Filled symbols indicate significant deviations of the post-PAS MEP amplitudes from baseline, asterisks indicate significant deviations of the drug vs PLC conditions with regard to identical time points (Student's *t* test, two-tailed, paired samples, $p < 0.05$).

motor cortex. Modification of the relative activation of D₁ and D₂ receptors by blocking the latter resulted in an abolition of iPAS-induced focal inhibitory plasticity. Further enhancement of relative D₁ activation by L-dopa under D₂ block reestablished focal and nonfocal inhibitory as well as excitatory plasticity. These results are in favor for a dose-dependent effect of D₁ activation on plasticity and deliver indirect evidence for the importance of balanced dopamine receptor activation for achieving a focusing dopaminergic effect on plasticity.

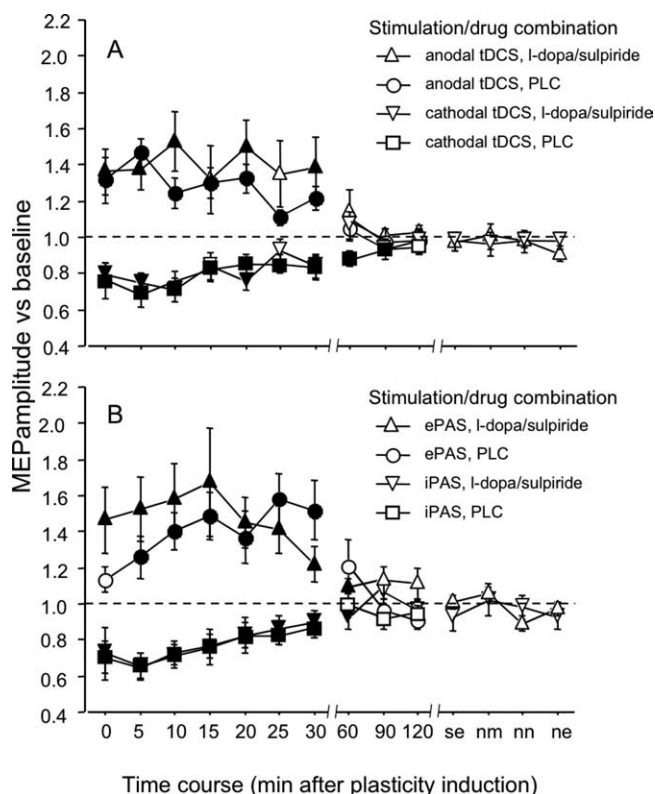


Figure 3. Impact of combined D₁ activation and D₂ block on plasticity induced by ePAS/and anodal or cathodal tDCS/iPAS (experiment 2). *A, B*, Shown are baseline-standardized MEP amplitudes after plasticity induction by anodal/cathodal tDCS (*A*) or ePAS/iPAS (*B*) under placebo medication or sulpiride and L-dopa up to the evening of the poststimulation day. In the placebo medication condition, anodal tDCS enhances excitability significantly for 30 min while cathodal tDCS results in an excitability reduction for 90 min. Whereas the anodal tDCS-induced excitability enhancement is not affected by sulpiride and L-dopa, these drugs reduce duration of the cathodal tDCS-generated excitability diminution. The iPAS- and ePAS-induced excitability modification was largely identical under placebo and real medication. Filled symbols indicate significant deviations of the post-tDCS MEP amplitudes from baseline (Student's *t* test, two-tailed, paired samples, $p < 0.05$).

The effect of relative D-receptor enhancement by D₂ block on plasticity

As shown by the results of experiment 1, blocking D₂ receptors, and thus shifting the relation of activity of D₁/D₂ receptors to the direction of D₁ receptors, but without enhancing absolute D₁-receptor activity, abolishes focal inhibitory plasticity, as induced by iPAS. Similarly, in a former experiment nonfocal inhibitory plasticity, induced by cathodal tDCS, was abolished by D₂-receptor block via sulpiride (Nitsche et al., 2006). This pattern of results is in accordance with an important influence of the D₂ receptor on inhibitory plasticity, as already shown for LTD in animals (Otani et al., 1998; Spencer and Murphy, 2000). Here, physiological D₁ activity without D₂ activity does not suffice to induce inhibitory plasticity.

In contrast, sulpiride did not extinguish, but slightly enhance the magnitude, of focal excitatory plasticity induced by ePAS. Interestingly, in another experiment sulpiride abolished the induction of nonfocal excitatory plasticity induced by anodal tDCS (Nitsche et al., 2006). Thus it seems that with regard to facilitatory plasticity, block of D₂ receptors has different effects dependent on the focality of plasticity induction. For the induction of focal plasticity, D₂ receptor involvement seems to be of no major importance. This result is in principal accordance with some animal experiments, showing a missing impact of D₂ receptors on LTP

(Gurden et al., 2000) but an important function of D₁ receptors (Otmakhova and Lisman, 1996; Chen et al., 1996; Bach et al., 1999; Bailey et al., 2000; Gurden et al., 2000; Huang et al., 2004). However, the slight, nonsignificant enhancement of the magnitude of the MEP amplitude increase under sulpiride, which might have become significant with larger subject numbers, might hint for a reducing effect of D₂ activity on ePAS-generated LTP-like plasticity. For nonfocal excitatory plasticity, as induced by anodal tDCS, however, under physiological D₁ activation D₂ activity seems to be essential for achieving the respective excitability enhancement. Since in another animal experiment weak, but not strong LTP was prevented by D₂ antagonism (Manahan-Vaughan and Kulla, 2003), this might indicate that the importance of the D₂ receptor differs with regard to the specific kind of excitatory plasticity induced.

Since sulpiride alone did not affect single pulse MEPs and baseline MEP amplitudes were identical between the respective experimental conditions, an impact of these factors on the results can be ruled out.

D₁-receptor activation under D₂-receptor block reestablishes plasticity

Adding L-dopa to sulpiride, and thus activating D₁ receptors absolutely under D₂ block, resulted in clearly different effects. Here, inhibitory plasticity induced by focal iPAS was reestablished, while facilitatory focal plasticity was preserved. Similarly, the effects of nonfocal (anodal/cathodal tDCS) stimulation protocols were reestablished, compared with the plasticity-abolishing effects of sulpiride in a foregoing experiment (Nitsche et al., 2006). Moreover, for both excitability-enhancing plasticity-inducing protocols, a slight, nonsignificant increase of MEP-amplitudes under the medication condition compared with PLC can be noticed, which might have become significant with larger subject numbers studied. On first sight, it might be argued that the L-dopa medication could have removed the D₂-receptor block and thus these effects are due to an activation of the whole dopaminergic system. However, L-dopa alone was demonstrated in a former study to affect plasticity, as induced by tDCS and PAS, in a different way: It turned the anodal tDCS-generated nonfocal excitability enhancement into inhibition, but prolonged nonfocal and focal inhibitory plasticity and focal excitatory plasticity (Kuo et al., 2008). This focusing effect is clearly not apparent in the present experiment. Moreover, the prolonging effect of L-dopa on plasticity without D₂ block has vanished, whereas a slight enlargement of the excitability-enhancing stimulation protocols occurs. This qualitatively different pattern of results is in favor for an effective D₁ activation and a still relevant D₂-receptor block accomplished by our medication. However, strictly speaking this is indirect evidence for an effective selective D₁ activation by this experimental protocol. Studies at the receptor level are needed to validate this design more directly.

Since baseline MEP amplitudes were identical between the respective experimental conditions, an impact of these factors on the results can be ruled out.

The inhibitory effect of D₁-receptor activity on acute motor cortex excitability is compatible with former animal experiments (Gorelova et al., 2002; Rosenkranz and Johnston, 2006; Kröner et al., 2007).

General remarks

The results of the current experiments in connection with those of former experiments of our group (Nitsche et al., 2006; Kuo et

al., 2008) shed light on the interaction of dopaminergic D₁ and D₂ receptors in producing a focusing effect on neuroplasticity.

Under physiological conditions, D₁-receptor activity does suffice for the induction of focal excitability-enhancing plasticity by brain stimulation, but additional D₂ activity is needed for the induction of nonfocal excitatory as well as inhibitory plasticity. This importance of D₂ receptors for the induction of plasticity can be overruled by increased D₁ receptor activation: enhancing D₁ receptor activity not only relative to D₂ activity, but by an absolute degree via L-dopa administration under D₂ receptor block reestablishes focal as well as nonfocal inhibitory and excitatory plasticity.

Interestingly, our experiments deliver indirect evidence for an importance of concomitant D₁ and D₂ activation on certain further aspects of plasticity. (1) D₁ activation alone does not exert a specific focusing effect on excitatory plasticity, as achieved by L-dopa medication in a former study (Kuo et al., 2008). Thus additional D₂ activity is needed to suppress the nonfocally induced excitability enhancement generated by anodal tDCS. At first sight, this is in opposition to the fact that D₂ block by sulpiride suppressed the anodal tDCS-induced excitability enhancement, but it fits to the fact that the combined D₁/D₂-, but predominant D₂-agonist pergolide slightly diminished the ability of anodal tDCS to enhance excitability (Nitsche et al., 2006). (2) Contrary to L-dopa alone, L-dopa combined with sulpiride did not prolong neuroplasticity. Under L-dopa, the effects of all plasticity protocols studied were relevantly stabilized in a former experiment (Kuo et al., 2008). In the current study, only a minor prolongation of the after-effects of facilitatory PAS was accomplished. Thus for consolidating neuroplasticity, a balanced D₁/D₂ activity seems necessary.

The results of the current study are in favor for an important impact of D₁ receptors on learning and memory formation via its supportive role with regard to the induction of neuroplastic network alterations. Accordingly, it was shown in animal experiments that D₁ receptor activation improves long-term memory (Seamans et al., 1998). Indirect evidence for a cognition-enhancing effect of D₁ activity is also available for humans. Application of a combined D₁/D₂ agonist and relative strengthening of D₁ receptor activity by D₂ receptor block enhanced performance in overlearned working memory tasks, while a selective D₂ agonist was without effect (Müller et al., 1998; Mehta et al., 2004). In contrast, the D₁/D₂ antagonist haloperidol impaired performance in a procedural learning task, but the selective D₂ antagonist sulpiride did not affect it (Paquet et al., 2004; Mehta et al., 2005). This, however, does not mean that D₂ activity is not important for or without effect on cognition in general. Indeed, the beneficial effect of dopaminergic subreceptors on cognition might be task-specific: D₁ activity might improve performance, when stable reactions, and thus respective plastic network alterations are needed. D₂ receptors might be beneficial for tasks that demand flexibility, where stable network modifications would be dysfunctional (Seamans and Yang 2004). Indeed, it has been shown by Mehta et al. (2004) that the D₂ antagonist sulpiride impaired performance in a working memory task requiring such flexible information processing. These different patterns of D₁ and D₂ effects on network function might also improve our understanding of dopaminergic dysfunction in neuropsychiatric diseases. In schizophrenia, it is suggested that distractibility, tangential and intrusive thought patterns are caused by an overactivity of D₂ receptors, and thus pathological enhanced “flexibility” of information processing (Seamans and Yang 2004). Consequently, blocking D₂ receptors and thus enhancing stability of information processing by relative enhancement of D₁ activation is the major therapeutic activity of anti-psychotic medication.

Together, the present experiments reveal distinct features of the D₁ receptor with regard to focal and nonfocal plasticity. Neuroplasticity, as tested here, is thought to be the neurophysiological basis of learning and memory formation. Since pharmacological intervention with different dopaminergic substances has been shown to result in specific modifications of plasticity, it might be worth to explore the property of specific dopaminergic drugs to enhance cognition in detail. Hereby, due to its unique focusing effect, it might come out that simultaneous D₁ and D₂ stimulation is better suited than stimulation of single subreceptors to enhance cognition. If so, this might have implications for the future treatment of patients with a dopaminergic dysfunction, accompanied by cognitive disturbances, e.g., patients with schizophrenia, Parkinson's, or Lewy body disease.

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