Catecholaminergic Consolidation of Motor Cortical Neuroplasticity in Humans

Amphetamine, a catecholaminergic re-uptake-blocker, is able to improve neuroplastic mechanisms in humans. However, so far not much is known about the underlying physiological mechanisms. Here, we study the impact of amphetamine on NMDA receptor-dependent long-lasting excitability modifications in the human motor cortex elicited by weak transcranial direct current stimulation (tDCS). Amphetamine significantly enhanced and prolonged increases in anodal, tDCS-induced, long-lasting excitability. Under amphetamine premedication, anodal tDCS resulted in an enhancement of excitability which lasted until the morning after tDCS, compared to −1 h in the placebo condition. Prolongation of the excitability enhancement was most pronounced for long-term effects; the duration of short-term excitability enhancement was only slightly increased. Since the additional application of the NMDA receptor antagonist dextromethorphan blocked any enhancement of tDCS-driven excitability under amphetamine, we conclude that amphetamine consolidates the tDCS-induced neuroplastic effects, but does not initiate them. The fact that propanolol, a β-adrenergic antagonist, diminished the duration of the tDCS-generated after-effects suggests that adrenergic receptors play a certain role in the consolidation of NMDA receptor-dependent motor cortical excitability modifications in humans. This result may enable researchers to optimize neuroplastic processes in the human brain on the rational basis of purpose-designed pharmacological interventions.

Keywords: adrenaline, amphetamine, propanolol, transcranial direct current stimulation, transcranial magnetic stimulation

Introduction

Learning depends critically on neuroplastic modifications in the efficacy of cortical neuronal processing. Long-lasting NMDA receptor-dependent cortical excitability and activity shifts, namely long-term potentiation (LTP), are involved in these processes (Rioult-Pedotti et al., 1998, 2000). In animals, dopaminergic and adrenergic mechanisms are important for consolidating and stabilizing NMDA receptor-dependent neuroplasticity (Ikegaya et al., 1997; Otani et al., 1998; Bailey et al., 2000) and learning (Stewart and Druhan, 1993).

Only indirect evidence for a similar process in the human brain is available so far. Amphetamine, which increases catecholaminergic transmitter availability, stabilizes use-dependent motor and improves sensory cortex plasticity (Bütefisch et al., 2002; Sawaki et al., 2002; Dinse et al., 2003), accelerates motor function recovery after stroke (Walker-Batson et al., 1995), improves learning and consolidation of verbal material (Soetens et al., 1993, 1995) and can increase performance in subjects with low working memory capacity (Mattay et al., 2000). However, it has not yet been tested in humans whether this is due to an indirect stabilization of NMDA receptor-induced neuroplasticity through increased intracerebral availability of catecholamines.

Here we use the transcranial administration of weak direct currents (tDCS), which results in excitability changes of motor and visual cortices in the human. These changes evolve during tDCS but are stable for up to an h after stimulation termination, if the duration of stimulation is sufficiently long. Anodal tDCS increases, while cathodal tDCS decreases excitability. These excitability changes are of intracortical origin (Nitsche and Paulus, 2000, 2001; Antal et al., 2003; Nitsche et al., 2005a). It was shown directly in the animal and by pharmacological intervention in the human that the initial effect of DC stimulation is accomplished by a hyper- or depolarization of neuronal membranes (Purpura and McMurtry, 1965; Nitsche et al., 2003b), whereas the after-effects seem to be NMDA receptor-dependent (Liebetanz et al., 2002; Nitsche et al., 2003b). Moreover, tDCS is of functional relevance: in the motor cortex it has been shown to modulate use-dependent neuroplasticity (Rosenkranz et al., 2000) and to improve implicit motor learning (Nitsche et al., 2003c).

The aim of this study was to test if tDCS-generated, neuroplastic excitability shifts can be consolidated by an increase of intracerebral catecholamine availability induced by oral premedication with amphetamine.

Therefore, in separate experimental sessions, the effect of amphetamine on: (a) cortical excitability shifts during short-lasting tDCS, which does not induce any after-effects; (b) short-lasting, tDCS-induced after-effects; and (c) long-lasting, tDCS-induced after-effects was studied for cathodal and anodal stimulation. A direct influence of tDCS on β-adrenergic activity was tested by applying protocol (c) under propanolol (PROP), an unselective β-adrenoceptor-antagonist. By combined administration of amphetamine and dextromethorphan (DMO, a non-competitive NMDA receptor-antagonist) in the case of anodal tDCS-generated long-term effects, we tested the selective role of amphetamine in stabilizing, but not inducing, the respective excitability shifts.

Materials and Methods

Subjects

Five to twelve healthy subjects participated in each experiment (for details see Table 1). Six of the subjects participated in all experiments and thus received six AMP exposures. All gave written informed consent. The investigation was approved by the ethics committee of the University of Goettingen and conformed to the Declaration of Helsinki.

Current Stimulation of the Motor Cortex

Direct currents were transferred by a saline-soaked pair of surface sponge electrodes (35 cm²) and delivered by a specially developed, battery-driven, constant current stimulator (Schneider Electronic, Michael A. Nitsche, Jessica Grundey, David Liebetanz, Nicolas Lang, Frithjof Tergau and Walter Paulus

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Gleichen, Germany) with a maximum output of 2 mA. The motor-cortical electrode was fixed over the representational field of the right abductor digiti minimi muscle (ADM) as determined by transcranial magnetic stimulation (TMS) and the other electrode contralaterally above the right orbit. In the different experiments, the currents flowed continuously for 4 s (excitability shifts during tDCS). 7 min (short-lasting after-effects), or 9 (cathodal tDCS) and 15 (anodal tDCS) min (long-lasting after-effects) with an intensity of 1.0 mA (current density ∼0.03 mA/cm²). These stimulation durations have been demonstrated to elicit the intended excitability shifts in excitability shifts in former experiments (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2003). Nearly all subjects were able to feel the current flow as a slight itching sensation at both the anodal and cathodal electrodes.

**Pharmacological Interventions**

Two hours before the start of each experimental session, 20 mg amphetamine (AMP), a precursor of amphetamine, which is completely metabolized into amphetamine (Honecker, 1975), 80 mg PROP, a combination of 20 mg AMP and 150 mg DMO or equivalent placebo (PLC) drugs were administered to the subjects orally (the different experiments are listed in Table 1). Two hours after oral intake, AMP, PROP and DMO were assumed to induce a stable plasma concentration, which was confirmed to elicit the intended durations in excitability shifts in former experiments (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2003; Bütefisch et al., 2002; Liebetanz et al., 2002; Nitsche et al., 2003b). To avoid cumulative drug effects, each experimental session was separated by at least 1 week. Subjects and the person conducting the experiment were blinded with regard to the patient's pharmacological condition, the drugs were administered by a different person.

**Measurement of Motor-cortical Excitability**

To detect current-driven changes of excitability, muscle-evoked potentials (MEPs) of the right ADM were recorded following stimulation of its motor-cortical representational field by single pulse TMS. These were induced using a Magstim 200 magnetic stimulator (Magstim Co., Whiteland, Dyfed, UK) and a figure-of-eight magnetic coil (diameter of one winding = 70 mm, peak magnetic field = 2.2 T). The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from midline. The optimal position was defined as the site where stimulation resulted consistently in the largest MEP. Surface EMG was recorded from the right ADM by use of 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. Signals were then digitized at an analogue-to-digital rate of 5 kHz and further relayed into a laboratory computer using the Neuroscan software collection (Neuroscan Inc., Herndon, VA) and conventional averaging software. The intensity of the stimulator output was adjusted for baseline recording so that the average stimulus led to an MEP of −1 mV, 5 min prior to DC stimulation.

**Experimental Procedures**

Each experiment was conducted in a repeated measurement design. The order of the conduction of the experiments was randomized.

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**Table 1**

| Stimulation paradigms, drug dosages and subject characteristics of the experiments |
|---------------------------------|-----------------|----------------|-----------------|-----------------|----------------|
| **DCS-condition**               | **Drug**        | **Dosage** (mg) | **TMS stimulation intensity (% of maximum stimulator output ± SD)** | **MEP amplitude (µV ± SD)** | **tDCS stimulation duration per cycle** |
| Intra-tDCS                      | AMP             | 20             | A: 53.33 ± 22.757                                              | A: 1008.30 ± 123.050        | 4s A/C          |
|                                 | PLC             | 40             | A: 54.00 ± 17.844                                              | A: 1008.13 ± 73.155         | 4s A/C          |
| Short-lasting after-effects     | AMP             | 20             | A: 43.83 ± 17.058                                              | A: 979.38 ± 116.280         | 7 min A/C       |
|                                 | PLC             | 40             | A: 43.66 ± 16.254                                              | A: 1012.08 ± 64.613         | 7 min A/C       |
| Long-lasting after-effects (a)  | AMP             | 20             | A: 41.89 ± 8.373                                               | A: 974.37 ± 68.434          | 13 min A        |
|                                 | PRO             | 80             | A: 42.75 ± 10.402                                              | A: 995.56 ± 43.956          | 13 min A        |
|                                 | PLC/PRO         | 40             | A: 44.83 ± 10.978                                              | A: 974.32 ± 83.899          | 13 min A        |
|                                 | PLC/AMP         | 40             | A: 43.89 ± 6.306                                               | A: 1031.11 ± 123.609        | 13 min A        |
|                                 | AMP/DMO         | 20/150         | A: 38.00 ± 7.649                                               | A: 1039.06 ± 61.833         | 13 min A        |
|                                 | PLC             | 40             | A: 41.60 ± 6.504                                               | A: 959.47 ± 13.044          | 13 min A        |

**tDCS condition** refers to the different experiments: intra-tDCS MEP measures (experiment 1), short-lasting (experiment 2) and long-lasting (experiment 3) after-effects. For each experiment, drugs and drug dosages are given. Mean TMS stimulation intensities to achieve non-tDCS (condition 1) and pre-tDCS (conditions 2 and 3) MEP amplitudes of −1 mV were calculated for each experimental condition. They did not differ between the respective drug/PLC conditions (Student’s t-test, P > 0.05). Also non-current (experiment 1) and pre-tDCS (experiments 2 and 3) MEP amplitude means were identical for the drug/PLC conditions (Student’s t-test, P > 0.05). tDCS stimulation duration was 4 s in experiment 1. Here, stimulation was repeated 15 times for each tDCS condition, whereas in the remaining protocols only one DC stimulation per session was applied. As shown in the last rows, six to twelve subjects participated in each experiment, and gender distribution was comparable between experiments. A, anodal tDCS; C, cathodal tDCS.
Effects of Amphetamine on tDCS-driven Excitability Shifts

With regard to cortical excitability changes during tDCS, the ANOVA revealed a significant main effect of tDCS (df = 3, F = 27.897, P < 0.001), but the main effect of the drug (df = 1, F = 0.695, P = 0.442) and interaction (df = 3, F = 1.507, P = 0.253) were not significant. As shown in Figure 1 and by the results of the t-tests, this is due to an excitability enhancement caused by anodal stimulation and an excitability reduction elicited by cathodal tDCS. These effects are identical in the AMP as well as in the PLC condition.

The ANOVA performed for the short-lasting after-effects revealed significant main effects of tDCS (df = 1, F = 111.665, P < 0.001) and time course (df = 7, F = 5.423, P < 0.001) and a significant interaction of current × time course (df = 7, F = 17.412, P < 0.001). The main effect of drug condition as well as the remaining interactions were not significant (P > 0.25). Anodal tDCS increased cortical excitability in the PLC and AMP condition, however, the effect lasted for 15 min in the AMP condition, but only for 10 min under PLC. The difference between AMP and PLC was significant for 15 min. Also, the after-effects of cathodal tDCS tended to last longer under AMP as compared to PLC; however, here the difference between both conditions proved not to be significant (Fig. 2).

With regard to the long-lasting after-effects in the AMP/PLC condition, the ANOVA revealed significant main effects of tDCS (df = 1, F = 47.170, P < 0.002) and time course (df = 9, F = 2.717, P = 0.016) as well as a significant interaction of both variables (df = 9, F = 12.683, P < 0.001). The main effect of drug condition as well as the remaining interactions were not significant (P > 0.1). As shown by the post hoc t-tests, anodal tDCS enhanced and cathodal tDCS diminished motor cortical excitability under AMP and PLC conditions. However, as compared to PLC, AMP prolonged the after-effects of anodal stimulation (30 min versus next morning) and decreased the duration and MEP amplitude shifts of the cathodal tDCS-induced excitability diminution. Hereby, the effects of AMP were much bigger and longer lasting with regard to the anodal tDCS condition. Whereas AMP prolonged the duration of the anodal stimulation-generated excitability enhancement until the morning.
short-lasting excitability enhancements. Shown are the mean ± SEM MEP amplitudes versus baseline across time following anodal or cathodal tDCS for AMP and placebo conditions. Fifteen minutes after anodal tDCS, AMP amplitudes are still enhanced under AMP, whereas under placebo medication they have reached baseline values. Asterisks indicate significant deviations of the post-tDCS MEP amplitudes from baseline values, crosses mark significant deviations of the respective AMP versus PLC conditions with regard to identical time points and tDCS polarities (Student’s t-test, two-tailed, paired samples, P < 0.05).

Figure 2. AMP prolongs tDCS after-effects after 7 min DC stimulation, which causes short-lasting excitability enhancements. Shown are the mean ± SEM MEP amplitudes versus baseline across time following anodal or cathodal tDCS for AMP and placebo conditions. Fifteen minutes after anodal tDCS, AMP amplitudes are still enhanced under AMP, whereas under placebo medication they have reached baseline values. Asterisks indicate significant deviations of the post-tDCS MEP amplitudes from baseline values, crosses mark significant deviations of the respective AMP versus PLC conditions with regard to identical time points and tDCS polarities (Student’s t-test, two-tailed, paired samples, P < 0.05).

Figure 3. AMP selectively prolongs the anodal tDCS after-effects of a 13 min stimulation period, which causes long-lasting excitability enhancements. The after-effects of cathodal tDCS are slightly reduced. Shown are the mean ± SEM MEP amplitudes versus baseline across time following anodal or cathodal tDCS for AMP and placebo conditions. On the morning following anodal tDCS, MEP amplitudes are still enhanced under AMP, whereas under placebo medication they have reached baseline values 60 min after tDCS. Asterisks indicate significant deviations of the post-tDCS MEP amplitudes from baseline values, crosses mark significant deviations of AMP versus PLC conditions with regard to identical time points and tDCS polarities (Student’s t-test, two-tailed, paired samples, P < 0.05). n.m., next morning; n.a., next afternoon; a, anodal tDCS; c, cathodal tDCS.

Discussion

The Amphetamine-induced Enhancements of Consolidation of tDCS-elicited Cortical Excitability Elevations are NMDA Receptor-dependent

In this study AMP selectively increased the duration of the anodal tDCS-induced motor cortical excitability enhancement as compared to PLC medication. This effect was relatively subtle for the short-lasting (7 min tDCS), but prominent for the long-lasting after-effects (9/13 min tDCS). It was restricted to the after-effects of anodal stimulation: tDCS eliciting no after-effects was not influenced by AMP. The necessary TMS output intensity to elicit MEP amplitudes of 1 mV magnitude was not modified by prior administration of AMP alone, thus a tDCS-independent effect of the drug on the amplitude of MEP can be ruled out. As suggested by a total loss of the after-effects from co-medication with DMO, a NMDA-antagonist, this effect is most likely caused by an NMDA receptor-dependent AMP-driven consolidation of the anodal tDCS-elicited cortical excitability enhancement and not due to a prominent direct effect of tDCS on catecholaminergic receptors.

36 % of the maximum stimulator output intensity was needed (SEM ±4.528 before AMP, ±5.115 after AMP intake, t = 0.000).

Effects of Propanol on tDCS-driven Excitability Modulations

For the long-lasting after-effects under PROP compared with PLC, the ANOVA revealed significant main effects due to the tDCS (df = 1, F = 39.876, P < 0.001) and time variables (df = 9, F = 2.352, P = 0.019) as well as a significant interaction of tDCS × time (df = 9, F = 21.760, P < 0.001). The main effect of drug condition and the remaining interactions were not significant (P > 0.089). According to the results of the post hoc t-tests, anodal as well as cathodal after-effects were relatively shortened under PROP as compared to the PLC condition. Whereas in the latter condition, anodal tDCS elicited after-effects lasting for at least 30 min and cathodal tDCS resulted in excitability shifts lasting 60 min, under PROP, both were restricted to 20 min after tDCS (Fig. 5).
Adrenaline may Contribute Relevantly to the Stabilization of Cortical Neuroplasticity

Since PROP, an unselective β-adrenergic antagonist, shortened the duration of the anodal tDCS after-effects, β-adrenergic receptor stimulation may be an important function of AMP for increasing consolidation of externally induced excitability enhancements. However, serotoninergic and dopaminergic contributions were not tested in this study. Since it is known that dopamine via the D1 receptor facilitates NMDA-dependent excitability and facilitates NMDA-dependent LTP through cAMP-dependent mechanisms, similar to what has been found for the β-adrenergic receptor in hippocampus (Otmakhova and Liman, 1996, 1998; Bailey et al., 2000), a relevant additional contribution by dopamine to these effects seems plausible. Moreover, it was shown that even a single administration of AMP can induce prominent and long-term enhancements of cortical dopamine signaling (Vanderschuren et al., 1999). In this way, a prolonged dopaminergic activation could have stabilized the tDCS-induced, NMDA receptor-dependent, excitability enhancements. On the behavioral level, AMP exposure leads to sensitization, which is the progressive and enduring enhancement of the psychomotor and positive reinforcing effects of the drug caused by its application (Stewart and Badiani, 1993). Sensitization seems to depend on D1 receptor and — at least partly — on NMDA receptor activity (Hu et al., 2002; Pacchioni et al., 2002). It thus shares certain similarities with the phenomenon of LTP and long-term memory formation. Thus it seems possible that AMP is able to improve long-term memory formation via a mechanism similar to behavioral sensitization. Further studies have to be conducted to test this hypothesis more specifically.

A tendency for AMP to prolong the short-lasting after-effects of excitability-diminishing cathodal tDCS was also noticed. This could also be due to increased adrenergic activity, because antagonizing adrenergic receptors with PROP shortened the after-effects of cathodal tDCS. Alternatively, dopaminergic effects are possible candidates, since dopamine is known to consolidate long-lasting neuronal excitability reductions in animals (Otani et al., 1998). However, this AMP-effect was not significant compared to the PLC condition and could not be repeated for the AMP long-term excitability diminution induced by cathodal tDCS. It can be speculated that different catecholaminergic receptors — all indirectly activated by application of AMP — are functioning antagonistically to the consolidation of motor cortical excitability diminutions and thus the effect of adrenergic activation on the net long-lasting cathodal after-effects is reduced. Alternatively, the tendency to reverse the effect of AMP on short-term and long-term after-effects may be due to different contributions of different receptors, depending on the time course of excitability changes. Additional experiments are needed to clarify this issue.

However, since PROP diminished the duration of the tDCS-induced excitability elevations and diminutions similarly, a certain amount of adrenergic activity seems to be necessary for the consolidation of both neuroplastic mechanisms.

General Remarks

These results add important information to the understanding of the mechanisms of consolidation of tDCS-induced neuroplasticity in the human motor cortex: catecholaminergic transmitters and here especially adrenergic ones seem to contribute relevantly and the catecholaminergic effects are NMDA receptor-dependent. This is in line with knowledge gained from animal experimentation (Ikegaya et al., 1997). The contribution of the dopaminergic system to the consolidation process, which is probable due to animal experimentation, has to be explored in future studies.

This specific action of AMP could explain its positive effects on learning and neuroplasticity in humans (Soetens et al., 1993, 1995; Walker-Bateson et al., 1995; Bütefisch et al., 2002; Sawaki et al., 2002). So far, knowledge about the effects of amphetamine on human cortical function was mainly phenomenological. The results of this study offer a neurophysiological explanation of its mode of action. In this way, they establish a basis for further studies exploring the possibly beneficial effects of AMP on cortical functions in humans more systematically. Here, the combination of AMP with other therapeutic strategies which induce neuroplasticity could be promising to stabilize their effects. For example, AMP could increase the efficacy of motor and visual training strategies applied after stroke to restitute compromised functions. Moreover, the efficacy of externally neuroplasticity-evoking techniques, e.g. TMS-induced excitability enhancements of the dorsolateral prefrontal cortex, which have been shown to be effective in depression, could possibly be enhanced by AMP. In this connection, it is important to note that AMP does not appear to have direct effects on cortical excitability as measured by TMS-evoked MEP, at least in the dosage applied here, but selectively consolidates them. This specific mode of action could be especially advantageous, since it may offer the possibility to selectively stabilize training- or stimulation-induced neuroplastic changes without inducing concurrent modifications, which could be maladaptive.

Notes

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References


