Modification of Practice-dependent Plasticity in Human Motor Cortex by Neuromodulators

Practice-dependent plasticity underlies motor learning in everyday life and motor relearning after lesions of the nervous system. Previous studies showed that practice-dependent plasticity is modifiable by neuromodulating transmitters such as norepinephrine (NE), dopamine (DA) or acetylcholine (ACh). Here we explored, for the first time comprehensively and systematically, the modifying effects of an agonist versus antagonist in each of these neuromodulating transmitter systems on practice-dependent plasticity in healthy subjects in a placebo-controlled, randomized, double-blind crossover design. We found that the agonists in all three neuromodulating transmitter systems (NE: methylphenidate; DA: cabergoline; ACh: tacrine) enhanced practice-dependent plasticity, whereas the antagonists decreased it (NE: prazosin; DA: haloperidol; ACh: biperiden). Enhancement of plasticity under methylphenidate and tacrine was associated with an increase in corticomotorneuronal excitability of the prime mover of the practice, as measured by the motor evoked potential amplitude, but with a decrease under cabergoline. Our findings demonstrate that agonists and antagonists in various neuromodulating transmitter systems produce significant and oppositely directed modifications of practice-dependent plasticity in human motor cortex. Enhancement of plasticity occurred through different strategies that either favoured extrinsic (NE, ACh) or intrinsic (DA) modulating influence on the motor cortical output network.

Keywords: acetylcholine, human motor cortex, monoamines, practice-dependent plasticity, transcranial magnetic stimulation

Introduction

Pyramidal neurons in the cerebral cortex transmit information largely via the excitatory neurotransmitter glutamate along intrinsic and cortico-cortical connections. Excitation is controlled by feedforward and feedback inhibition, mediated by inhibitory interneurons via the neurotransmitter gamma-aminobutyric acid (GABA). In addition, cortical function is strongly influenced by neuromodulating transmitters like norepinephrine (NE), dopamine (DA) or acetylcholine (ACh). These systems have in common that their axons originate from nuclei in the brainstem and project to all regions and layers of cortex (Cooper et al., 2002). While broad evidence exists on profound effects of neuromodulating transmitters on excitability and plasticity in sensory cortices (Gu, 2002) their effects on motor cortex have been studied less extensively. The motor cortex is a highly modifiable structure (Sanes and Donoghue, 2000) and repeated practice or skill learning are associated with substantial representational plasticity (Pascual-Leone et al., 1995; Nudo et al., 1996; Klein et al., 1998; Liepert et al., 1999). ACh is important for practice-dependent motor cortical plasticity in rats because lesions of the basal forebrain cholinergic system disrupt cortical map reorganization and impair motor skill acquisition (Conner et al., 2003).

In humans, practice-dependent motor cortical plasticity can be studied non-invasively by various transcranial magnetic stimulation (TMS) protocols (Classen and Cohen, 2003). One particularly well-established protocol included subjects in whom it was possible to induce with focal TMS of one motor cortex isolated thumb movements of the contralateral hand pointing consistently into one direction. If subjects then practiced brisk thumb movements into the opposite direction, thumb movements induced by TMS shifted into the direction of the trained movements after the end of practice (Classen et al., 1998). This suggested rapid changes in the cortical representation of the thumb, which encode kinematic details of the practiced movement and may reflect a first step towards skill acquisition. This form of practice-dependent plasticity is significantly reduced by dextromethorphan, an N-methyl-D-aspartate (NMDA) receptor blocker, and by lorazepam, a positive allosteric modulator of the GABA_A receptor (Bütefisch et al., 2000), suggesting that long-term potentiation like mechanisms are involved. Several previous studies already tested the effects of single drugs affecting neuromodulating neurotransmission on this form of practice-dependent plasticity. It was found that d-amphetamine, an indirect agonist of NE, DA and serotonin, and levodopa, a precursor of DA enhanced practice-dependent plasticity (Bütefisch et al., 2002; Sawaki et al., 2002b; Floel et al., 2005a) while the antiadrenergic α1 receptor antagonist prazosin (Sawaki et al., 2003) and the anticholinergic non-selective muscarinic receptor antagonist scopolamine (Sawaki et al., 2002a) reduced it. These reports suggest that neuromodulating transmitters exert controlling influence on practice-dependent plasticity in human motor cortex. However, no study has systematically tested agonists versus antagonists in the same neuromodulating transmitter system. Therefore, the major aim of the present study was to explore the effects of one agonist versus one antagonist in the NE (methylphenidate versus prazosin), DA (cabergoline versus haloperidol) and ACh system (tacrine versus biperiden) on practice-dependent motor cortical plasticity of healthy subjects in a placebo-controlled, randomized and double-blind crossover study design.

It is likely that the results of this study are pertinent to practice-dependent plasticity in clinical settings like motor relearning during stroke rehabilitation (Goldstein, 1998). The few available studies suggest a beneficial role of NE and DA agonists but the evidence is rather anecdotal due to small patient sample sizes (Crisostomo et al., 1988; Grade et al., 1998; Walker-Batson et al., 1995, 2001; Scheidtmann et al., 2001) and not unanuimous (Treig et al., 2003). One recent study showed that a single oral dose of levodopa enhanced practice-dependent...
plasticity in chronic stroke patients when examined in the present thumb movement practice protocol (Floel et al., 2005b).

Materials and Methods

Subjects

Eight healthy right-handed subjects (mean age 25.5 ± 4.6 years, six women) were included into the study. All subjects gave written informed consent, and the study was approved by the local ethics committee of the JW Goethe University hospital of Frankfurt, Germany. All experiments conformed to the Declaration of Helsinki. Altogether 40 subjects were initially screened but only the eight subjects selected for this study fulfilled all electrophysiological inclusion criteria (resting motor threshold < 50% of maximum stimulator output, isolated and consistent thumb movement elicited by TMS). The inclusion criteria were more extensive (threshold criterion) and more strict (quantified consistency criterion of TMS evoked thumb movements, see below for details) than in previous experiments (Classen et al., 1998; Büttetsch et al., 2000, 2002). This explains the lower inclusion rate in this study (20%) compared with the other studies (45-83%).

Recording and Stimulation

Subjects were seated in a comfortable chair. Their dominant right arm was adducted in the shoulder, 90° flexed in the elbow, and the semi-pronated forearm, the wrist and the four long fingers were immobilized in a cast while the thumb was entirely unconstrained (Fig. 1A). Thumb movements were recorded by two uni-axial accelerometers (Model 2256A-100, voltage sensitivity 100 mV/g, Endevco Corp., San Juan Capistrano, CA) that were mounted on the proximal phalanx of the thumb in orthogonal planes to detect acceleration in the abduction-adduction and extension-flexion axes, respectively (Fig. 1A). The raw signal was amplified (Model 133 signal conditioner, Endevco), digitized (A/D rate 4 kHz, Micro 1401, Cambridge Electronic Design, Cambridge, UK) and bandpass filtered (0.05–70 Hz, Spike 2 software, Cambridge Electronic Design). In addition, electromyography (EMG) was recorded by bipolar Ag-AgCl surface electrodes from two thumb muscles, the abductor pollicis brevis (APB) and the flexor pollicis brevis (FPB) (Fig. 1A). The EMG raw signal was amplified and bandpass filtered (0.5–2 kHz, Counterpoint Mk2 Electromyograph, Dantec, 2740 Skovlund, Denmark) and digitized (A/D rate 4 kHz, Micro 1401).

TMS of the hand area of the left primary motor cortex was achieved by means of a figure-of-eight stimulating coil (outer diameter of each wing, 90 mm) connected to a Magstim 200 magnetic stimulator with a monophasic current waveform (The Magstim Company Ltd, Carmarthen, UK). The coil was held tangential to the scalp with the handle pointing backwards and ~45° away from the midline. This is the optimal orientation for activating the corticospinal system transsynaptically via horizontal cortico-cortical connections (Di Lazzaro et al., 2004).

Time Line of an Experimental Session

The stimulating coil was moved in 1 cm steps around the suspected site of the hand area of the left motor cortex to define the stimulation site that produced consistently largest motor evoked potentials (MEPs) in the APB and FPB of the right hand. Then resting motor threshold (RMT) was determined at this site in decremental steps of 1% maximum stimulator output. RMT was defined as the minimum stimulus intensity that elicited MEPs > 50 μV in the APB in at least 5 of 10 consecutive trials (Rossini et al., 1999). Subjects had to meet an RMT < 50% of the maximum stimulator output to be eligible for further testing because higher thresholds indicate insufficient accessibility of the hand area of the motor cortex by TMS. If a subject was tested for the first time, additional inclusion criteria were checked in the next step: one
requirement was that it had to be possible to evoke isolated thumb movements by TMS without overt movements of any of the other digits, wrist or arm; the other requirement was that the direction of TMS-induced thumb movements was sufficiently consistent (Bütefisch et al., 2000). Consistency was tested before drug intake (PRE1, Fig. 1B) by obtaining 40 TMS-induced thumb movements (inter-trial interval, 10 s). Acceleration vectors were constructed from the first-peak acceleration measured by the two accelerometers in the orthogonal axes of abduction-adduction and extension-flexion axes. All acceleration vectors were then assigned a length of 1 in a unity circle defined by the abduction-adduction and extension-flexion axes. Dispersion of the TMS-induced thumb movement vectors is then given by the length of the mean vector, and the requirement of sufficient consistency was fulfilled if the dispersion was >0.7.

Immediately after PRE1, the subject took one of the seven study drugs (see below). Two hours later, another block of 40 trials of TMS-induced thumb movements was obtained (PRE2, Fig. 1B). PRE2 served as the baseline for calculation of practice-dependent plasticity during (comparison of measurements D1-D5 with PRE2) and after the end of practice (comparison of measurements P1-P6 with PRE2), and to analyse whether there were any drug effects on TMS-induced thumb movements before practice (comparison between PRE2 and PRE1).

Motor practice consisted of six blocks of 300 thumb movements each, paced by a metronome at a rate of 1 Hz (duration of each block, 5 min). Subjects were instructed to perform phasic thumb movements as fast and brief as possible into the direction exactly opposite to (i.e. 180° away from) the direction of the mean vector of TMS-induced thumb movements obtained during PRE2. These six practice blocks (T1-T6, Fig. 1B) were interspersed with five blocks of TMS-induced thumb movements (D1-D5), each consisting of 10 trials (inter-trial interval, 10 s) in order to obtain information on how fast practice-dependent plasticity developed during practice. After the end of practice, another 6 blocks of TMS-induced thumb movements (P1-P6) were recorded, each block consisting of 20 trials (inter-trial interval, 10 s), in order to obtain information on the duration of practice-dependent plasticity for the first 30 min after the end of practice (Fig. 1B).

All recordings of TMS-induced thumb movements were obtained during full voluntary relaxation that was monitored by continuous audio-visual EMG feedback at high gain (50 μV/D). Trials that were contaminated by EMG activity were rejected online and discarded from further analysis.

### Analysis of Kinematic and MEP Data

Final analysis of the accelerometer data was performed offline by programs written by the authors (U.E.) in Matlab Version 6.1 (The MathWorks, Inc., Natick, MA). TMS-induced thumb movements during PRE1 and PRE2 were analysed by amplitude (length of the mean acceleration vector, in m/s²) and dispersion (see above). Voluntary thumb movements during motor practice were recorded by accelerometry during all blocks of practice (T1-T6, Fig. 1B). From these data practice performance was evaluated by analysing amplitude (length of the mean acceleration vector across all blocks of practice T1-T6, in m/s²), dispersion and angle deviation of the mean vector of voluntary thumb movements from the mean vector of TMS-induced thumb movements during PRE2 (ΔPHI, in degrees). Practice-dependent plasticity was analysed in two ways: (i) increase of the percentage of TMS-induced thumb movements into the training target zone (TTZ), which was defined by a zone of ±30° around the direction of the mean acceleration vector of practiced thumb movements (Classen et al., 1998; Bütefisch et al., 2000); and (ii) angle deviation of the mean vector of TMS-induced thumb movements during (D1-D5) and after motor practice (P1-P6) from the mean vector of TMS-induced thumb movements during baseline (PRE2, ΔPHI, in degrees). Practice-dependent plasticity was evaluated by the following two methods: (i) increase of the percentage of TMS-induced thumb movements into the TTZ, weighted PHI) were analysed by two-way repeated-measures ANOVAs (ANOVA) with Drug as within-subject factor. Drug effects on practice-dependent plasticity (percentage increase into TTZ, weighted PHI) were analysed by one-way repeated-measures ANOVAs with Drug as within-subject factor. Drug effects on practice-dependent plasticity were tested in a placebo-controlled, randomized and double-blind crossover design. Each subject was tested for all six study drugs and placebo in different sessions. Each drug was applied as a single oral dose. The minimum interval between consecutive sessions in a given subject was one week to avoid interaction between drugs or sessions. Main modes of action, doses and pharmacokinetics of the study drugs are given in Table 1. The single oral doses equalled typical daily doses for treatment of patients, and/or have already demonstrated efficacy in previous TMS experiments to alter motor cortical excitability or plasticity (Table 1).

### Statistics

Differences between drugs regarding kinematics (amplitude, dispersion) of TMS-induced thumb movements at baseline (PRE2) were evaluated by one-way repeated-measures analyses of variance (ANOVAs), with Drug as within-subject factor. Drug effects on kinematics (amplitude, dispersion) of TMS-induced thumb movements were evaluated by ANOVAs with Drug as within-subject factor. Differences between drugs regarding the training performance (amplitude, dispersion and ΔPHI of the mean training vector) were analysed by one-way repeated-measures ANOVAs with Drug as within-subject factor. Drug effects on practice-dependent plasticity (percentage increase into TTZ, weighted PHI) were analysed by two-way repeated-measures ANOVAs with Drug as within-subject factor.

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### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Main mode(s) of action</th>
<th>Dose (mg)</th>
<th>Plasma peak (h)</th>
<th>Previous TMS studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (PBO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin (PZA)</td>
<td>α1 receptor antagonist</td>
<td>1 mg</td>
<td>2</td>
<td>Sawaki et al. (2003)</td>
</tr>
<tr>
<td>Methylphenidate (MPH)</td>
<td>NE receptor antagonist</td>
<td>40 mg</td>
<td>2</td>
<td>Illic et al. (2003)</td>
</tr>
<tr>
<td>Haloperidol (HAL)</td>
<td>D2 receptor antagonist</td>
<td>2.5 mg</td>
<td>2-6</td>
<td>Ziemann et al. (1997)</td>
</tr>
<tr>
<td>Clobazam (CAB)</td>
<td>D2 receptor antagonist</td>
<td>2 mg</td>
<td>2 (0.5-4)</td>
<td>Unpublished own observations</td>
</tr>
<tr>
<td>Biperiden (BIP)</td>
<td>M1 receptor antagonist</td>
<td>8 mg</td>
<td>1.5</td>
<td>Unpublished own observations</td>
</tr>
<tr>
<td>Tacrine (TAC)</td>
<td>Acetylcholinesterase inhibitor</td>
<td>40 mg</td>
<td>1.5</td>
<td>Korchev et al. (2005)</td>
</tr>
</tbody>
</table>
Results

Two out of eight subjects dropped out of the study due to adverse drug effects (both subjects experienced nausea and vomiting under BIP). The remaining six subject tolerated the drugs well and were capable of fully complying with all aspects of the experiments. All reported data are based on these six subjects.

**RMT and Stimulus Intensities Used for TMS-induced Thumb Movements**

There was no difference between Drugs with respect to RMT before drug intake \[F(6,30) = 0.91, P = 0.50\]. The grand mean of RMT across all sessions and subjects was \(4.2 \pm 5.3\%\) of maximum stimulator output. There was also no difference between Drugs with respect to the TMS intensity used to induce thumb movements before drug intake \[F(6,30) = 1.15, P = 0.36\]. The grand mean of TMS intensity across all sessions and subjects was \(47.3 \pm 2.2\%\) of maximum stimulator output. RMT and TMS intensity remained unchanged 2 h after drug intake (PRE2). Therefore, there were no differences between Drugs in stimulus intensity that could have accounted for the dissociating drug effects on practice-dependent plasticity (see below).

**Characteristics of TMS-induced Thumb Movements before Training**

Grand averages across all drugs of TMS-induced thumb movements at time point PRE2 (before training) revealed a mean dispersion of \(0.83 \pm 0.06\) and a mean percentage into the TTZ of \(12 \pm 28\%\), indicating that these movements were consistent into one direction and that movements into the TTZ only very rarely occurred in about \(1\%\) of the trials. Drugs had no significant effect on dispersion \[F(6,30) = 0.62, P = 0.71\], percentage into the TTZ \[F(6,30) = 0.60, P = 0.72\] or amplitude \[F(6,30) = 2.14, P = 0.078\] at time point PRE2 (Table 2). There were also no differences in characteristics of TMS-induced thumb movements [dispersion: \(F(6,30) = 1.11, P = 0.38\); percentage into TTZ: \(F(6,30) = 0.77, P = 0.60\); amplitude: \(F(6,30) = 1.10, P = 0.39\)] when comparing PRE2 (2 h after drug intake) with PRE1 (immediately before drug intake). Therefore, it can be concluded that there were no consistent drug effects on TMS-induced thumb movements before training that accounted for the effects of drug on practice-dependent plasticity.

**Training Performance**

Drugs had no effect on dispersion \[F(6,30) = 1.05, P = 0.41\] and ΔPHI \[F(6,30) = 1.17, P = 0.35\] of voluntary thumb movements during training (Table 3). There was a slight effect of Drug on the amplitude of voluntary thumb movements \[F(6,30) = 2.64, P = 0.036\], but post hoc tests showed no significant difference of any of the drugs with placebo (Table 3). Thus, there were no consistent drug effects on training performance that accounted for the effects of drugs on practice-dependent plasticity.

**Practice-dependent Plasticity – Increase of TMS-induced Thumb Movements into the TTZ**

The two-way ANOVA across all time points during and after training (D1–P6) revealed a significant effect of Drug \[F(6,30) = 5.16, P = 0.0009\]. Post hoc comparisons of the single drugs with PBO showed that MPH (\(P < 0.001\)), CAB (\(P < 0.001\)) and TAC (\(P = 0.001\)) resulted in an increase of practice-dependent plasticity, while HAL (\(P = 0.001\)) and BIP (\(P = 0.007\)) resulted in a decrease (Fig. 2 A–D). Since the two-way ANOVA also showed a significant interaction between Drug and Time \[F(60,300) = 1.48, P = 0.019\], additional ANOVAs were run that were restricted to the time points either during training (D1–D5) or after training (P1–P6). During training, there were significant effects of Drug \[F(6,30) = 5.84, P = 0.0004\] and Time \[F(4,20) = 4.01, P = 0.015\], while the interaction between Drug and Time was not significant \[F(24,120) = 0.83, P = 0.70\]. Post hoc comparisons of the single drugs with PBO revealed that practice-dependent plasticity occurred more rapidly under MPH (\(P = 0.0001\)) and CAB (\(P = 0.0002\)), but developed to a lesser extent under HAL (\(P = 0.023\)) and BIP (\(P = 0.025\)). In addition, practice-dependent plasticity was non-significantly enhanced under TAC (\(P = 0.052\)) (Fig. 2A–C,E). After the end of training, there was a significant effect of Drug \[F(6,30) = 3.41, P = 0.011\] and the interaction between Drug and Time \[F(30, 150) = 2.04, P = 0.0028\]. Post hoc comparisons of the single drugs with PBO showed that the gain in practice-dependent plasticity observed during training was retained for under CAB (\(P < 0.0001\)) and TAC (\(P = 0.01\)) but not under MPH (\(P = 0.12\)). Practice-dependent plasticity remained less under HAL (\(P = 0.014\)) when compared with practice-dependent learning under PBO (Fig. 2A–C,F).

**Practice-dependent Plasticity – wPHI**

Analysis of practice-dependent plasticity by increase of TMS-induced thumb movements into the TTZ is an all-or-none procedure that has the potential disadvantage of missing small training induced changes in the direction of TMS-induced thumb movements. Therefore, an analysis of wPHI (see Materials and Methods) was also performed. The two-way ANOVA across all time points during and after training (D1–P6) revealed a significant effect of Drug \[F(6,30) = 9.67, P < 0.0001\]. This was explained in the post hoc tests by an enhancement of practice-dependent plasticity under MPH (\(P < 0.0001\), CAB

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**Table 2** Characteristics of TMS-induced thumb movements before training (PRE2)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dispersion</th>
<th>% into TTZ</th>
<th>Amplitude (m/s²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBO</td>
<td>0.86 ± 0.06</td>
<td>0 ± 0</td>
<td>0.80 ± 0.17</td>
</tr>
<tr>
<td>PRZ</td>
<td>0.83 ± 0.04</td>
<td>2.1 ± 0.9</td>
<td>1.11 ± 0.69</td>
</tr>
<tr>
<td>MPH</td>
<td>0.80 ± 0.07</td>
<td>0 ± 0</td>
<td>1.30 ± 0.62</td>
</tr>
<tr>
<td>HAL</td>
<td>0.84 ± 0.07</td>
<td>2.8 ± 1.1</td>
<td>1.40 ± 0.99</td>
</tr>
<tr>
<td>CAB</td>
<td>0.86 ± 0.05</td>
<td>2.0 ± 0.8</td>
<td>1.28 ± 0.95</td>
</tr>
<tr>
<td>BIP</td>
<td>0.84 ± 0.07</td>
<td>4.6 ± 1.9</td>
<td>0.80 ± 0.43</td>
</tr>
<tr>
<td>TAC</td>
<td>0.83 ± 0.09</td>
<td>4.5 ± 1.9</td>
<td>0.80 ± 0.16</td>
</tr>
</tbody>
</table>

All data are means ± SD from six subjects.

**Table 3** Training performance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dispersion</th>
<th>ΔPHI (°)</th>
<th>Amplitude (m/s²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBO</td>
<td>0.91 ± 0.02</td>
<td>171.3 ± 11.0</td>
<td>5.3 ± 2.8</td>
</tr>
<tr>
<td>PRZ</td>
<td>0.87 ± 0.04</td>
<td>173.5 ± 7.7</td>
<td>6.5 ± 2.0</td>
</tr>
<tr>
<td>MPH</td>
<td>0.89 ± 0.03</td>
<td>169.8 ± 10.6</td>
<td>4.7 ± 2.5</td>
</tr>
<tr>
<td>HAL</td>
<td>0.90 ± 0.03</td>
<td>166.7 ± 3.2</td>
<td>6.3 ± 3.8</td>
</tr>
<tr>
<td>CAB</td>
<td>0.91 ± 0.04</td>
<td>156.7 ± 45.7</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>BIP</td>
<td>0.89 ± 0.05</td>
<td>159.1 ± 11.6</td>
<td>5.3 ± 1.4</td>
</tr>
<tr>
<td>TAC</td>
<td>0.91 ± 0.03</td>
<td>146.7 ± 35.0</td>
<td>5.8 ± 2.8</td>
</tr>
</tbody>
</table>

All data are means ± SD from six subjects.
(P < 0.0001) and TAC (P = 0.0003) but a suppression under HAL (P = 0.0003) and BIP (P < 0.0001) (Fig. 3A–D). Restriction of the ANOVA to the time points during training (D1–D5) demonstrated significant effects of Drug [F(6, 30) = 7.83, P < 0.0001] and Time [F(4, 20) = 4.38, P = 0.011]. The post hoc tests revealed that practice-dependent plasticity occurred more rapidly under MPH (P < 0.0001), CAB (P < 0.0001) and TAC (P = 0.0068), but developed to a lesser extent under BIP (P = 0.002) (Fig. 3A–C). Finally, restriction of the ANOVA to the time points after the end of training (P1–P6) showed again a significant effect of Drug [F(6, 30) = 4.71, P = 0.0017]. This was explained in the post hoc tests by a retention of the gain in practice-dependent plasticity under CAB (P < 0.0001) and TAC (P = 0.022) but not MPH (P = 0.23), while practice-dependent plasticity remained low under BIP (P = 0.0038) and declined away from the PBO level under PRZ (P = 0.020) and HAL (P = 0.0009) (Fig. 3A–C, F).

EMG Data

During PRE2, the grand mean MEP amplitude across all sessions and subjects in the prime mover of the motor practice was 0.96 ± 0.76 mV, and there were no differences between Drugs (P = 0.23). MEP amplitudes during and after practice (D1–P6) normalized to the MEP amplitude during baseline (PRE2) increased, as indicated by a significant effect of Time in the two-way ANOVA [F(10, 50) = 4.12, P = 0.0004] while there were no significant effects of Drug (P = 0.17) or the interaction between Drug and Time (P = 0.45) (Fig. 4). For some drugs the time course of changes in MEP amplitude showed similarity with the time course of practice-dependent plasticity (e.g. for PBO and MPH in Figs 2A and 4A) while for other drugs the time course was clearly different (e.g. CAB in Figs 2B and 4B). In order to get more insight into the relation between changes in MEP amplitude and practice-dependent plasticity during
training, regression analyses were conducted for those drugs that enhanced practice-dependent plasticity (MPH, CAB, TAC). All regressions were significant but relations were quite different: while it was logarithmic for MPH, it was inverse linear for CAB and normal linear for TAC (Fig. 5). Regression analyses were not significant for any of the other drugs ($P > 0.05$).

**Discussion**

The present experiments combine previously reported results on pharmacological modulation of practice-dependent plasticity in a single systematic double-blind placebo-controlled crossover design. The major finding is that agonists and antagonists of the main neuromodulating transmitters systems (norepinephrine, dopamine, acetylcholine) produce significant and oppositely directed modifications of practice-dependent plasticity in human motor cortex. The agonists of all three systems (methylphenidate, cabergoline, tacrine) enhanced practice-dependent plasticity, while the antagonists (prazosin, haloperidol, biperiden) reduced it.

**Mechanisms of Practice-dependent Motor Cortical Plasticity**

We investigated practice-dependent plasticity by means of a standardized TMS protocol (Classen et al., 1998). The site of plasticity, i.e. the practice-induced increase of TMS-induced thumb movements into the training target zone, is very likely in the motor cortex because the post-training angular deviation of thumb movements elicited with transcranial electrical stimulation (TES) was significantly smaller compared with thumb movements elicited with TMS (Classen et al., 1998). The implication is that TES activates the corticomotoneuronal system predominantly directly rather than transsynaptically (Day et al., 1989). Therefore, a plasticity effect visible with
TMS but not TES points to a site upstream of the corticomotoneuronal axon. Previous experiments showed that this form of practice-dependent plasticity was blocked by the NMDA receptor antagonist dextromethorphan (Bütefisch et al., 2000). This led to the idea that a long-term potentiation (LTP)-like strengthening of excitatory synapses in motor cortical circuits was involved (Bütefisch et al., 2000) because LTP is an important mechanism of plasticity (Sanes and Donoghue, 2000) that, in the motor cortex, depends on the activation of NMDA receptors (Hess et al., 1996). Furthermore, this form of practice-dependent plasticity was prevented by the benzodiazepine lorazepam, an allosteric positive modulator at the GABA_A receptor (Bütefisch et al., 2000). This indirectly supported the LTP hypothesis further because disinhibition through local application of a GABA_A receptor antagonist facilitated LTP in motor cortical slice experiments (Hess and Donoghue, 1994; Castro-Alamancos et al., 1995; Hess et al., 1996). In summary, the available data are compatible with the idea that this form of practice-dependent plasticity as studied in the present experiments relies on LTP-like strengthening of synaptic efficacy. The following paragraphs discuss, in this context of an LTP-dependent process, some of the basic mechanisms that might underlie the observed modifying effects of agonists and antagonists in the three neuromodulating transmitter systems.

**Modification of Practice-dependent Plasticity in the Norepinephrine System**

NE enhanced LTP evoked by theta-burst stimulation in slices of rat visual cortex by increasing the depolarizing response to the tetanus, and in turn, by increasing NMDA receptor-gated conductances during this response (Bröcher et al., 1992). The authors suggested that this mode of action accounts for the facilitatory effects of NE on use-dependent synaptic plasticity in the visual cortex (Bear and Singer, 1986). In rat motor cortex, NE increased the excitability of large pyramidal cells in layer V through a reduction of slow K^{+} currents, and through an increase of the persistent inward Na^{+} current (Foehring et al., 1989). It is likely that these NE effects are capable of promoting LTP in motor cortex but this has not been tested yet in slice experiments.

In humans, MPH increases corticomotoneuronal excitability and decreases GABAergic intracortical inhibition (Ilic et al., 2003). Both effects may have been involved in the significant enhancement of practice-dependent plasticity by MPH observed in the present study. It is very likely that this enhancement of plasticity is at least in part mediated through the α1 receptor because the α1 receptor antagonist PRZ reduced practice-dependent plasticity in the present experiments and in one previous study (Sawaki et al., 2003) while the non-selective β-blocker propranolol showed only a clearly weaker and non-significant trend towards reduction of practice-dependent plasticity (Sawaki et al., 2003).

**Modification of Practice-dependent Plasticity in the Dopamine System**

In monkeys, the motor cortex receives the greatest density of dopaminergic fibres of all cortical areas (Lewis et al., 1987; Williams and Goldman-Rakic, 1993). A prominent effect of iontophoretic application of DA to cortical neurons is to depress their activity (Krnjevic and Phillis, 1963). This effect is most likely mediated by dopaminergic D2 receptors (Gulledge and Jaffe, 1998). In vitro studies in human cortical tissue showed that DA reduced neuronal excitability by decreasing neurotransmission through non-NMDA glutamate receptors (Cepeda et al., 1992). In contrast, DA increased neurotransmission through NMDA receptors (Cepeda et al., 1992). This important study strongly supports the idea that DA is capable of enhancing the signal-to-noise ratio in a neuronal network by suppressing unwanted inputs (noise) but strengthening pathways that conduct repeated and consistent inputs (Huda et al., 2001). Therefore, it is not a paradox that LTP
short-interval intracortical facilitation, a measure of the excitability of fast glutamatergic input to motor cortical output neurons, and by decreasing short-interval intracortical inhibition (Korchoumov et al., 2005). In accord with these data, the present experiments showed that TAC facilitated practice-dependent plasticity whereas BIP suppressed it. BIP is a selective M1 receptor antagonist. Therefore, the present findings confirm and extend one previous study that reported a suppression of practice-dependent plasticity under the non-selective M1/M2 receptor antagonist scopolamine (Sawaki et al., 2002a).

**Time Course of Neuromodulating Effects on Practice-dependent Plasticity**

Our findings (Figs 2 and 3) show that the enhancement of practice-dependent plasticity developed similarly during training under MPH, CAB and TAC, and was maintained for at least 30 min after the end of training under CAB and TAC but not under MPH. Only one previous study had explored the time course of a drug modulating effect on this form of practice-dependent plasticity after the end of training (Bütefisch et al., 2002). It was found that d-amphetamine resulted in a long-lasting enhancement (>60 min). Since d-amphetamine results in a strong increase in extracellular DA concentration (Kuczenski and Segal, 2001), those data and the present CAB data are in close agreement. The time courses of depressant effects on practice-dependent plasticity by PRZ, HAL and BIP also differed slightly. Interference with the development of practice-dependent plasticity during training was most conspicuous under BIP while after the end of training all three drugs resulted in less plasticity than under PBO. Mechanisms of these differences in time courses of practice-dependent plasticity modulation are unclear at present. It is also unknown whether the different time courses have any practical relevance, but it may be speculated that they favour DA and ACh over NE if long-lasting facilitation of training effects is desired, as during neurorehabilitation of stroke patients.

**Relation between Motor Cortical Excitability and Practice-dependent Plasticity**

The regression analysis between practice-dependent changes in MEP amplitude of the prime mover muscle of the practice and practice-dependent plasticity (wPHI) demonstrated that the relation between corticomotoneuronal excitability and practice-dependent plasticity is not uniform (Fig. 5).

dependent processes such as practice-dependent plasticity are enhanced by DA although neuronal excitability as determined by fast excitatory post-synaptic potentials mediated through non-NMDA glutamate receptors is decreased.

In accord with these studies at the cellular level, TMS experiments at the systems level of human motor cortex showed that the D2 receptor agonist CAB significantly depressed the MEP intensity curve, while the D2 receptor antagonist HAL increased it (U. Ziemann et al., unpublished observations). The MEP intensity curve is a measure of corticomotoneuronal excitability, reflecting TMS excitation of extrinsic inputs to motor cortex through cortico-cortical axons and non-NMDA glutamate receptors (Amassian et al., 1987; Di Lazzaro et al., 2003). The present experiments are the first to show that, dissociated from the effects of CAB vs. HAL on corticomotoneuronal excitability, CAB enhanced practice-dependent plasticity while HAL depressed it. These findings are in line with the enhancing effect of levodopa on practice-dependent plasticity (Floel et al., 2005a). Since dopamine is an agonist at both the D1/D5 and D2 receptor families, it is not clear whether these receptors were differentially involved in the plasticity modulating effect of levodopa (Floel et al., 2005a).

The present experiments specify that the D2 receptor plays a significant role in this form of motor cortical plasticity.

**Modification of Practice-dependent Plasticity in the Acetylcholine System**

ACh facilitates, through activation of muscarinic receptors, in particular the M1 receptor, pyramidal cell discharge in neocortex and the responsiveness of these cells to excitatory inputs (McCormick and Prince, 1986; Schwindt et al., 1988; Cox et al., 1994). This cholinergic modulation is associated with enhancement of neurotransmission through the NMDA receptor (Bröcher et al., 1992). Accordingly, in slice experiments of rat motor cortex, pharmacological blockade of muscarinic receptors by atropine prevented the induction of LTP after theta-burst stimulation but rather promoted the induction of long-term depression (Hess and Donoghue, 1999). ACh is also important for practice-dependent motor cortical plasticity in rats because lesions of the basal forebrain cholinergic system disrupted cortical map reorganization and impaired motor skill acquisition (Conner et al., 2003).

In human motor cortex, the ACh-esterase inhibitor TAC shifts the balance from inhibition to excitation by increasing short-interval intracortical facilitation, a measure of the excitability of fast glutamatergic input to motor cortical output neurons, and by decreasing short-interval intracortical inhibition (Korchoumov et al., 2005). In accord with these data, the present experiments showed that TAC facilitated practice-dependent plasticity whereas BIP suppressed it. BIP is a selective M1 receptor antagonist. Therefore, the present findings confirm and extend one previous study that reported a suppression of practice-dependent plasticity under the non-selective M1/M2 receptor antagonist scopolamine (Sawaki et al., 2002a).
practice-dependent plasticity under CAB was associated with a decrease. A proper assessment of the APB and FDI acting as agonists vs. antagonists of the motor practice was not possible due to the problem of volume conduction between the EMG recordings, and because in some instances when thumb movements were practiced in abduction-flexion direction, both muscles were training agonists. However, this does not limit data interpretation because even when information of MEP amplitudes of true agonists and antagonists of the practice was available in previous studies, it was generally found that the ratio of MEP amplitudes of agonists over antagonists was not closely linked with the magnitude of practice-dependent plasticity as measured by the increase of TMS-induced thumb movements into the training target zone (Bütefisch et al., 2000, 2002; Floel et al., 2005a). This strongly suggests that an increase in corticomotorneuronal excitability of the agonist of motor practice is neither sufficient (Floel et al., 2005a) nor necessary (present data) to explain the practice-dependent shift in the direction of TMS-induced thumb movements. The question arises of how it is possible, as observed under CAB, that enhancement of practice-dependent plasticity is associated with a decrease in MEP amplitude of the training agonist. One possibility is that an even stronger decrease in excitability of corticomotorneuronal representations of the training antagonists occurred, leading to an improved signal-to-noise ratio in favour of the training agonist.

The contrasting properties observed here with respect to the relation of MEP amplitude and practice-dependent plasticity under MPH and TAC (normal relation) versus CAB (inverse relation) are compatible with the effects of these drugs on corticomotorneuronal cell activity per se and the responsiveness of these cells to external input: MPH and TAC increase it, while CAB decreases it (see above). This suggests that enhancement of practice-dependent plasticity by neuromodulating transmitting agents may occur through fundamentally different strategies that either favour extrinsic (NE, ACh) or intrinsic (DA) modulating influence on the motor cortical output network (Hasselmo, 1995). Whether one of these strategies is more effective in supporting motor rehabilitation in the clinical setting is unknown yet, as it was shown in lesion experiments in animals (for a review, see Feeney and Sutton, 1987) and in humans after stroke that antagonists in all three neuromodulating transmitter systems facilitate sensorimotor recovery (Crisostomo et al., 1988; Walker-Batson et al., 1995, 2001; Grade et al., 1998; Scheidtmann et al., 2001; Floel et al., 2005b) while antagonists impede it (for a review, see Goldstein, 1998).


