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Neuromodulatory Neurotransmitters Influence LTP-Like Plasticity in Human Cortex: A Pharmaco-TMS Study

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Long-term potentiation (LTP) of synaptic efficacy is considered a fundamental mechanism of learning and memory. At the cellular level a large body of evidence demonstrated that the major neuromodulatory neurotransmitters dopamine (DA), norepinephrine (NE), and acetylcholine (ACh) influence LTP magnitude. Noninvasive brain stimulation protocols provide the opportunity to study LTP-like plasticity at the systems level of human cortex. Here we applied paired associative stimulation (PAS) to induce LTP-like plasticity in the primary motor cortex of eight healthy subjects. In a double-blind, randomized, placebo-controlled, crossover design, the acute effects of a single oral dose of the neuromodulatory drugs cabergoline (DA agonist), haloperidol (DA antagonist), methylphenidate (indirect NE agonist), prazosine (NE antagonist), tacrine (ACh agonist), and biperiden (ACh antagonist) on PAS-induced LTP-like plasticity were examined. The antagonists haloperidol, prazosine, and biperiden depressed significantly the PAS-induced LTP-like plasticity observed under placebo, whereas the agonists cabergoline, methylphenidate, and tacrine had no effect. Findings demonstrate that antagonists in major neuromodulatory neurotransmitter systems suppress LTP-like plasticity at the systems level of human cortex, in accord with evidence of their modulating action of LTP at the cellular level. This provides further supportive evidence for the known detrimental effects of these drugs on LTP-dependent mechanisms such as learning and memory.
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INTRODUCTION

Long-term potentiation (LTP) of synaptic efficacy in neocortical networks is considered a fundamental mechanism of learning and memory formation (Asanuma and Pavlides, 1997; Sanes and Donoghue, 2000; Lynch, 2004; Feldman, 2009). At the cellular level, the neuromodulatory neurotransmitters dopamine (DA), norepinephrine (NE), and acetylcholine (ACh) can significantly influence the expression of LTP (Gu, 2002, 2003; Otani *et al*, 2003). Recently developed noninvasive brain stimulation protocols provide the opportunity to study LTP-like plasticity at the systems level of human cortex (Cooke and Bliss, 2006; Thickbroom, 2007; Ziemann *et al*, 2008; Müller-Dahlhaus *et al*, 2010).

With respect to the physiological properties, paired associative stimulation (PAS) is the currently best investigated of these protocols (Ziemann *et al*, 2008; Müller-Dahlhaus *et al*, 2010). Electrical peripheral nerve stimulation is repeatedly paired with transcranial magnetic stimulation (TMS) of the contralateral motor cortex. If the interstimulus interval is adjusted so that arrival of the afferent stimulus in motor cortex coincides with or slightly precedes TMS, then this typically leads to long-term increase of motor cortical excitability as measured by motor evoked potential (MEP) amplitude. The duration of MEP increase is 30–60 min minimum but reversible within 24 h (Stefan *et al*, 2000). The MEP increase is dose dependent, that is, its magnitude and duration scales with the number of stimulus pairs (Nitsche *et al*, 2007). It saturates at ∼160–170% (Stefan *et al*, 2004; Nitsche *et al*, 2007). The site of MEP increase is in the motor cortex because motor responses elicited by direct electrical stimulation of the corticospinal tract do not change (Stefan *et al*, 2000), whereas epidural recordings of the descending corticospinal volley at the level of the cervical spinal cord show a significant increase (Di Lazzaro *et al*, 2009). Finally, pharmacological blockade of *N*-methyl-D-aspartate receptors (NMDARs) prevents the PAS-induced MEP increase (Stefan *et al*, 2002). In summary, these findings provide convergent evidence that the PAS-induced long-term increase in MEP amplitude can be taken as a model of LTP-like plasticity at the systems level of human motor cortex (Cooke and Bliss, 2006; Ziemann *et al*, 2008; Müller-Dahlhaus *et al*, 2010). This is supported further by the

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significant interactions of PAS with LTP-dependent processes such as motor learning (Ziemann *et al*, 2004; Stefan *et al*, 2006; Rosenkranz *et al*, 2007; Jung and Ziemann, 2009; Kang *et al*, 2011).

Pharmacological modulation of PAS-induced LTP-like plasticity is a relatively little explored field and the available data have not always been consistent. In the dopaminergic system, levodopa enhances its magnitude and duration (Kuo *et al*, 2008) but no longer when D2 receptors are blocked by sulpiride (Nitsche *et al*, 2009). On the other hand, the D2 receptor agonist ropinirole decreases PAS-induced LTP-like plasticity dose dependently in an inverted U-shaped manner (Monte-Silva *et al*, 2009). In the cholinergic system, the cholinesterase inhibitor rivastigmine strongly increases magnitude and duration of PAS-induced LTP-like plasticity (Kuo *et al*, 2007), whereas nicotine results in nonsignificant prolongation but no change in magnitude (Thirugnanasambandam *et al*, 2011). Studies in the noradrenergic system have not been done. Here we explored systematically the effects of neuromodulatory drugs (NMDs), that is, agonists and antagonists in all three major neuromodulatory neurotransmitter systems (DA, NE, and ACh), in a double-blind, randomized, placebo-controlled crossover design in healthy subjects. We expected significant modulating effects on PAS-induced LTP-like plasticity. These findings are pertinent to the setting of clinical neurorehabilitation, where NMDs may have detrimental or beneficial effects on the long-term outcome of sensorimotor function in stroke patients (Goldstein, 1995; Ziemann *et al*, 2006).

SUBJECTS AND METHODS

Subjects

A total of 24 right-handed (Oldfield, 1971) healthy, drug-naive subjects (age range 18–32 years; 11 women) were screened for resting motor threshold (RMT) of $\leqslant$50% of maximum stimulator output, and for PAS-induced LTP-like increase of MEP amplitude $\geqslant$1.2 (ratio of MEP post-PAS/pre-PAS) using a previously established PAS protocol (Stefan *et al*, 2000, 2002). LTP-like plasticity is highly variable between subjects (Müller-Dahlhaus *et al*, 2008; Ridding and Ziemann, 2010), but RMT $\leqslant$50% is a quick indicator for a likely ‘PAS responder’ (Müller-Dahlhaus *et al*, 2008). Like in other studies (Heidegger *et al*, 2010), a minimum amount of LTP-like plasticity of 1.2 was required because the primary aim of this study was test drug modulation of LTP-like plasticity. In all, 8 subjects (age range 19–26 years; 3 women) met the inclusion criteria and were enrolled into the study. All subjects gave written informed consent before participation. The study was approved by the ethics committee of the Goethe-University Hospital of Frankfurt and conforms to the latest version of the Declaration of Helsinki Principles.

Electromyography (EMG)

Surface EMG was recorded from the right abductor pollicis brevis (APB), using Ag-AgCl cup electrodes in a belly–tendon montage. The EMG raw signal was amplified and filtered (0.02–2 kHz; Counterpoint Mk2 electromyograph; Dantec, Skovlunde, Denmark), digitized (analog–digital rate, 5 kHz; CED Micro 1401; Cambridge Electronic Design, Cambridge, UK), and fed into a laboratory computer for online visual display and offline analysis. All recordings were obtained during muscle rest, which was monitored audio-visually using high-gain EMG (50 μV/division).

Transcranial Magnetic Stimulation

Focal TMS was delivered through a figure-of-eight coil (diameter of each wing, 70 mm) connected to a Magstim 200 magnetic stimulator with a monophasic current waveform (The Magstim Company, Carmarthenshire, Wales, UK). The coil was held tangential to the scalp with the handle pointing backwards and 45° away from the midline so that the current induced in the brain ran from lateral-posterior to medial-anterior. This is the optimal orientation for transsynaptic activation of the corticospinal system (Di Lazzaro *et al*, 2008). The coil was held over the hand area of the left primary motor cortex (M1), defined as the optimal site for eliciting MEP in the right APB . This site was marked on the scalp with a felt-tip pen to assure a stable coil placement throughout the experiment. The RMT was determined as the minimum stimulus intensity that elicited a small MEP of $\geqslant$50 μV in at least 5 out of consecutive 10 trials in the voluntarily relaxed right APB (Rossini *et al*, 1999).

Induction of LTP-Like Plasticity by PAS

PAS consisted of 90 stimulus pairs delivered over a period of 30 min at a rate of 0.05 Hz according to an established protocol (Stefan *et al*, 2000, 2002). Bipolar electrical stimulation of the right median nerve at the wrist (cathode proximal, constant-current square pulses of 1 ms duration, intensity of three times the perceptual sensory threshold) preceded TMS of the hand area of the left M1 by the individually determined latency of the median nerve somatosensory evoked early cortical potential (N20) plus 2 ms. This interstimulus interval resulted in previous studies in consistent and reproducible LTP-like plasticity, that is, a long-lasting ($>$30 min) on average 1.5-fold increase in MEP amplitude (Müller *et al*, 2007; Jung and Ziemann, 2009; Heidegger *et al*, 2010). The TMS intensity was adjusted to elicit on average peak-to-peak MEP amplitudes of 1 mV ($MEP_{1\,mV}$) when TMS was given alone.

Attention Level

The level of attention, a significant modulator of PAS effects (Stefan *et al*, 2004), was controlled by asking the subjects to watch the stimulated hand and count the total number of electrical stimuli applied to the right median nerve during PAS. In addition, immediately before PAS, subjects rated their level of sedation on an ordinal scale, with 0 meaning no, 1 mild, 2 moderate, and 3 strong sedation.

Study Drugs

The acute drug effects on PAS-induced LTP-like plasticity were assessed for a single oral dose of the six NMDs in Table 1 and placebo (PBO). The NMD doses were selected

Table 1 Study Drugs

Drug	Main mode(s) of action	Dose (mg)	Plasma peak (h)
Placebo (PBO)			
Cabergoline (CAB)	Dopamine (D2) receptor agonist	2 mg	2 (0.5–4)
Haloperidol (HAL)	Dopamine (D2) receptor antagonist	2.5 mg	2–6
Methylphenidate (MPH)	Indirect NE (and DA) agonist	40 mg	2
Prazosine (PRZ)	α1-Adrenergic receptor antagonist (NE antagonist)	1 mg	2
Tacrine (TAC)	Cholinesterase inhibitor (ACh agonist)	40 mg	1.5
Biperiden (BIP)	M1 muscarinic receptor antagonist (ACh antagonist)	8 mg	1.5

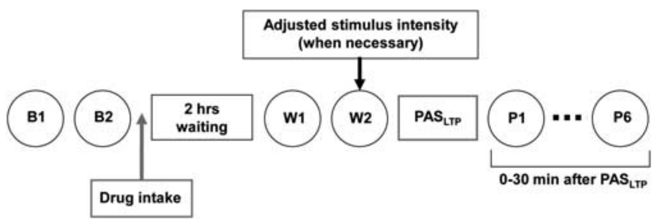

Figure 1 Time line of experimental procedures. The circles indicate blocks of 20 trials of MEP amplitude measurements (B1, B2: baseline before drug intake; W1, W2: 2 h after drug intake and immediately before PAS$_{LTP}$; P1–P6: 0–30 min after PAS$_{LTP}$). At B1, B2, and W2, TMS intensity was adjusted to elicit MEP amplitudes of on average 1 mV. The ratio W1/B informed on drug-induced change in MEP amplitude, whereas the ratio P1–P6/W2 informed on PAS-induced MEP change.

because they equal typical daily doses in clinical usage and/or have already been demonstrated to alter significantly TMS measures of motor cortical excitability (for reviews, see Ziemann, 2004; Paulus *et al*, 2008; for specific references, see Table 1). The main modes of NMD action and their pharmacokinetics are also summarized in Table 1. Of note, all NMDs reach peak plasma levels approximately 2 h after oral intake.

Study Design

The six NMDs and PBO were given in separate sessions in a double-blind crossover design. The order of drugs was pseudo-randomized and counterbalanced across subjects. The intersession interval in a given subject was at least 1 week to exclude drug interference and carryover effects (Heidegger *et al*, 2010).

The time line of a single session is shown in Figure 1. All sessions started with two baseline blocks (B1, B2) of 20 MEP trials. The intertrial interval varied randomly between 8 and 12 s to minimize anticipation of the next trial. TMS intensity was adjusted to elicit $MEP_{1\,mV}$. The study drug was taken immediately after B2. After a waiting period of 2 h (to reach NMD plasma peaks), another two blocks of 20 MEPs were recorded (W1 and W2). The measurements in W1 in comparison with baseline were used to assess NMD effects on corticospinal excitability. If the mean MEP amplitude in W1 deviated by $>30\%$ from the mean of the MEP amplitudes in B1 and B2, TMS intensity during W2 was adjusted to re-establish $MEP_{1\,mV}$. This adjustment of TMS intensity was necessary in two subjects after prazosine (PRZ) and in two subjects after biperiden (BIP). This procedure assured that MEP amplitude was similar across drug conditions at the start of PAS for induction of LTP-like plasticity (Heidegger *et al*, 2010). Then, PAS was applied as described above. MEP amplitude after PAS was measured in six blocks (P1–P6), covering the first 30 min after PAS in steps of 5 min. Each block consisted of 20 trials using the same stimulus intensity as in W2.

Statistics

All MEP data were checked for normal distribution using the Kolmogorov–Smirnov test. As normal distribution was confirmed throughout, parametric statistics (analysis of variance, ANOVA) were applied.

Motor Cortical Excitability Before Drug Intake

MEP amplitudes were averaged over the baseline time points B1 and B2. Baseline MEP amplitudes were compared between drugs (between-subject effect, seven levels: six NMDs and PBO) using ANOVA.

Drug Effects on Motor Cortical Excitability

MEP amplitudes at time point W1 were normalized to B (average of B1 and B2). The effects of drug (between-subject effect, seven levels: six NMDs and PBO) on the MEP ratio W1B was assessed using ANOVA. Because there was a main effect of drug on the MEP ratio W1B (see Results), *post hoc* paired *t*-tests adjusted for multiple comparisons using Bonferroni's method were conducted to compare the single drug conditions with PBO. Another ANOVA was calculated on the MEP ratio W2/B to ensure that, after TMS intensity adjustment, drug (between-subject effect, seven levels: six NMDs and PBO) no longer had an effect on MEP amplitude.

Drug Effects on PAS-Induced LTP-Like Plasticity

The primary measure of PAS-induced LTP-like plasticity was the mean MEP amplitude obtained during P1–P6 normalized to the mean MEP amplitude at time point W2. The effects of drug on PAS-induced LTP-like plasticity were analyzed in a repeated-measures ANOVA (rmANOVA) with the within-subject effect of time (six levels: P1–P6) and the between-subject effect of drug (seven levels: six NMDs and PBO). Because there was a main effect of DRUG (see Results), six *post hoc* pairwise comparisons of PAS-induced LTP-like plasticity under the single NMD *vs* PBO were performed using rmANOVAs with the within-subject effects of drug (two levels, NMD *vs* PBO) and time (six levels, P1–P6). Adjustment for multiple comparisons was applied using Bonferroni's method.

For all tests, significance was assumed if $P<0.05$. Data are reported as means ± 1 SEM.

In addition, drug effects on PAS-induced LTP-like plasticity were evaluated by calculating effect size, using Cohen's *d* (Cohen, 1988). Beyond statistical significance, Cohen's *d* estimates the biological relevance of these effects. Absolute values of Cohen's $d<0.8$ indicate weak or moderate effect sizes, whereas Cohen's $d \geqslant 0.8$ indicates strong effect sizes.

RESULTS

In one subject, the cabergoline (CAB) session had to be terminated after recording of W2 due to nausea and vomiting. Otherwise, all subjects tolerated the experimental procedures well. One subject noted slight sedation (level 1 on the ordinal scale 0–3) in the haloperidol (HAL) session and two subjects in the BIP session, whereas no sedation (level 0) was rated in all other sessions. All subjects were capable of maintaining full compliance with all requirements of the tasks.

Motor Cortical Excitability Before Drug Intake

The $MEP_{1\,mV}$ amplitudes before drug intake (mean MEP amplitude of time points B1 and B2) were not different between drugs ($F_{6,42}=1.57$, $P=0.18$), and were always close to the targeted amplitude of 1 mV: PBO 1.05 ± 0.18 mV; CAB 1.06 ± 0.09 mV; HAL 1.17 ± 0.11 mV; methylphenidate (MPH) 1.06 ± 0.12 mV; PRZ 1.07 ± 0.09 mV; tacrine (TAC) 1.04 ± 0.06 mV; BIP 1.07 ± 0.09 mV.

Drug Effects on Motor Cortical Excitability

The effect of drug on MEP amplitude (W1 normalized to B) was significant ($F_{6,42}=3.43$, $P=0.008$). *Post hoc* paired *t*-tests showed that PRZ increased MEP amplitude when compared with PBO ($P=0.008$), whereas other drugs had no significant effect (Figure 2a). After adjustment of TMS intensity, the effect of drug on MEP amplitude (W2 normalized to B) remained borderline significant ($F_{6,42}=2.34$, $P=0.047$), but the *post hoc* comparisons showed that the MEP ratio W2/B was no longer significantly different for any NMD compared with PBO (Figure 2b). This is an important nil finding because there were no differences in MEP amplitude immediately before PAS that could have accounted for the significant drug effects on PAS-induced LTP-like plasticity (see below).

Drug Effects on PAS-Induced LTP-Like Plasticity

In the PBO condition, PAS resulted in a significant LTP-like increase in MEP amplitude (MEPs averaged across time points P1–P6 normalized to MEP amplitude at time point W2; 1.71 ± 0.05, $P<0.001$, one-sample *t*-test; Figure 3).

The rmANOVA revealed a significant effect of drug on PAS-induced LTP-like plasticity ($F_{6,36}=11.59$, $P=0.0004$, Figure 3), whereas there were no significant effects of time ($F_{5,30}=1.55$, $P=0.21$) or of the interaction of drug and time ($F_{30,180}=0.81$, $P=0.75$). *Post hoc* pairwise comparisons of PAS effects of each NMD with PBO revealed that induction of LTP-like plasticity was significantly reduced after intake of HAL ($P<0.0001$; MEPs averaged across time points P1–P6 normalized to MEP amplitude at time point W2, 1.04 ± 0.03), PRZ ($P<0.0001$; MEP_{P1-P6}/MEP_{W2} 1.04 ± 0.04), and BIP ($P=0.0007$; MEP_{P1-P6}/MEP_{W2} 1.20 ± 0.05). All other pairwise comparisons with PBO were not significant ($P>0.1$, Figure 3). One-sample *t*-tests revealed that significant LTP-like increases in MEP amplitude occurred for CAB ($P<0.001$), MPH ($P<0.001$), and TAC ($P=0.03$), whereas this was not the case for HAL, PRZ, and BIP (all $P>0.05$, Figure 3).

Calculation of effect size using Cohen's *d* for the pairwise comparisons of PAS effects (MEP_{P1-P6}/MEP_{W2}) under the influence of each NMD *vs* PBO revealed the following values: CAB *vs* PBO: $d=0.63$; HAL *vs* PBO: $d=-2.63$; MPH *vs* PBO: $d=0.13$; PRZ *vs* PBO: $d=-2.49$; TAC *vs* PBO: $d=-0.59$; BIP *vs* PBO: $d=-1.72$. Only the suppressive effects of HAL, PRZ, and BIP reached values of $|d| \geqslant 0.8$, indicating strong effect sizes.

DISCUSSION

The key novel findings of this study are that antagonists of major neuromodulatory neurotransmitter systems (DA, NE,

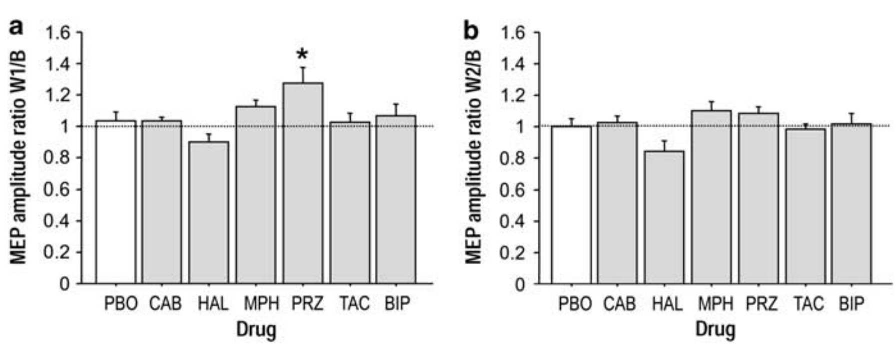

Figure 2 (a) MEP amplitude changes induced by the drugs (*x* axis, PBO: placebo; CAB: cabergoline; HAL: haloperidol; MPH: methylphenidate; PRZ: prazosine; TAC: tacrine; BIP: biperiden), expressed as ratio W1/B (*y* axis). B denotes the average of MEP recordings at baseline recordings B1 and B2. (b) MEP amplitude changes after correction of TMS intensity expressed as ratio W2/B. The horizontal dotted lines indicate 1.0, that is, no change in MEP amplitude. All data are means ($n=8$) + 1 SEM. *$p<0.05$.

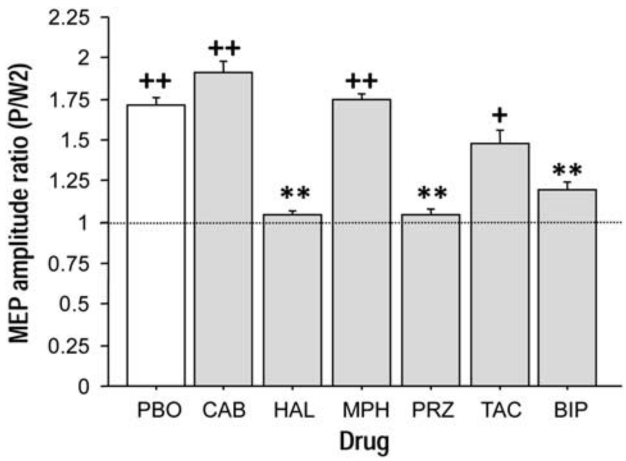

Figure 3 Effects of drugs (*x* axis, PBO: placebo; CAB: cabergoline; HAL: haloperidol; MPH: methylphenidate; PRZ: prazosine; TAC: tacrine; BIP: biperiden) on PAS-induced LTP-like plasticity expressed as MEP amplitude ratio P1–P6/W2 (*y* axis). The horizontal dotted line indicates 1.0, that is, no change in MEP amplitude. Note that PAS resulted in an LTP-like increase by 1.71 ± 0.05 in the PBO condition (white bar), whereas HAL, PRZ, and BIP led to significant depressions, and CAB, MPH, and TAC had no modulating effect when compared with PBO. All data are means ($n = 8$ for all drug conditions except CAB, where only 7 subjects completed the session) + 1 SEM. **$p < 0.001$ (two-tailed paired *t*-test drug *vs* PBO); $^{+}p < 0.05$; $^{++}p < 0.001$ (one-tailed *t*-tests indicating difference from 1.0).

and ACh) led to strong reductions of PAS-induced long-term increase in MEP amplitude, a model of LTP-like plasticity at the systems level of human cortex, whereas the effects of agonists in these neuromodulatory systems were nonsignificant. The single findings are discussed in the following paragraphs.

Drug Effects on Motor Cortical Excitability

Measurements of motor cortical excitability were restricted to $MEP_{1\,mV}$ because the primary focus of this study was to examine modulating drug effects on PAS-induced LTP-like plasticity. The effects of agonists or antagonists of the major neuromodulatory neurotransmitter systems on MEP amplitude have not been studied widely in the past (for review, see Ziemann, 2004; Paulus *et al*, 2008). The effects were by and large weak and inconsistent, with the exception of NE agonists that produced a significant increase in MEP amplitude in most of the studies. The absence of major drug-induced MEP changes in this study ($MEP_{W1/B}$, Figure 2a) is in accord with the literature. This is an important nil finding because the drug effects on PAS-induced LTP-like plasticity occurred in the absence of significant drug influence on MEP amplitude, the primary measure of LTP-like plasticity. The absence of relevant drug effects on corticomotor excitability *per se* and previous convergent evidence that PAS-induced LTP-like plasticity occurs at the site of the sensorimotor cortex (Stefan *et al*, 2000; Di Lazzaro *et al*, 2009) renders it very likely that the observed drug effects on PAS-induced plasticity occurred specifically at the level of sensorimotor cortex, even though the drugs were given systemically.

Drug Effects on PAS-Induced LTP-Like Plasticity

Dopaminergic, noradrenergic, and muscarinergic receptors are broadly represented in monkey and human M1 (Huntley *et al*, 1992; Geyer *et al*, 1996; Kötter *et al*, 2001), supporting a critical modulating role of these neuromodulatory neurotransmitter systems in motor function. Studies on the modulating effects of these neurotransmitter systems on LTP in M1 are, however, very scarce: the dopamine D1 receptor antagonist SCH02339 and the dopamine D2 receptor antagonist raclopride decrease LTP in rat M1 (Molina-Luna *et al*, 2009). Pharmacological blockade of muscarinic receptors by atropine also prevents the induction of LTP and rather favors the induction of long-term depression by the same stimulation protocol (Hess and Donoghue, 1999). Studies on a possible enhancement of LTP in M1 by neuromodulatory neurotransmitters are, to the best of our knowledge, not available.

We used here the PAS-induced LTP-like increase in MEP amplitude as a surrogate for LTP at the systems level of human motor cortex. We are fully aware that the evidence for this proposition is circumstantial but, given that the characteristics of the PAS-induced MEP increase are in all known detail consistent with LTP at the cellular level (see Introduction), this has become a widely accepted proposition even by cellular physiologists (Cooke and Bliss, 2006; Müller-Dahlhaus *et al*, 2010).

The significant drug effects on PAS-induced LTP-like plasticity were all suppressive and were caused by HAL, PRZ, and BIP, the antagonists of the examined neuromodulatory neurotransmitter systems ($MEP_{P1-P6/W2}$, Figure 3). Given the reported beneficial effects of agonists in these systems on motor learning and sensorimotor outcome after cerebral stroke (see below), one might have expected that CAB, MPH, and TAC had resulted in enhancing effects on PAS-induced LTP-like plasticity. However, a critical appraisal of the existing literature on pharmacological modulation of PAS-induced plasticity does not support this expectation: sulpiride, a selective dopamine D2 receptor antagonist, results in slight (nonsignificant) enhancement of PAS-induced LTP-like plasticity (Nitsche *et al*, 2009), whereas ropinirole, a dopamine D2/D3 receptor agonist, dose dependently leads to a reduction (Monte-Silva *et al*, 2009). Furthermore, global dopamine receptor (ie, D1 and D2 receptor family) activation by levodopa, a precursor of dopamine, increases magnitude and duration of PAS-induced LTP-like plasticity (Kuo *et al*, 2008), but only in the absence of dopamine D2 receptor blockade by sulpiride (Nitsche *et al*, 2009). These findings imply that a balanced co-activation of dopamine D1 and D2 receptors is necessary to enhance PAS-induced LTP-like plasticity. The absence of an enhancement of LTP-like plasticity by the selective dopamine D2 receptor agonist CAB in the present study is exquisitely consistent with those previous data.

The absence of an enhancement of PAS-induced LTP-like plasticity by TAC is at first sight surprising, given that a single oral dose of 3 mg of the brain-selective cholinesterase inhibitor rivastigmine resulted in clear increase of magnitude and duration of this form of LTP-like plasticity (Kuo *et al*, 2007). Thus, 40 mg of TAC and 3 mg of rivastigmine are the typical daily starting doses and are equivalent to 25% of the recommended maximum daily dose in the treatment of Alzheimer's disease. The TAC/rivastigmine single oral dose ratio to result in 50% inhibition of brain cholinesterase inhibition in rats is ~5.6 (Kosasa *et al*, 2000). As the TAC/rivastigmine dose ratio in the present *vs* previous study (Kuo *et al*, 2007) is 13.3, it is highly unlikely that a too low dose of TAC explains the lack of its effect on PAS-induced LTP-like plasticity. One potentially important difference between the two drugs relates to their differential potency of *decreasing* electrically evoked ACh release through presynaptic muscarinic receptor-mediated autoinhibition. Although this is not observed to any measurable extent after acute exposure of human brain slices by rivastigmine, autoinhibition of ACh release by TAC occurs at brain concentrations that are likely reached by therapeutic doses (Jackisch *et al*, 2009). In the present experimental setting, the electrical peripheral nerve stimulation is associated with activation of central cholinergic afferents (Di Lazzaro *et al*, 2000; Tokimura *et al*, 2000). Therefore, it may be speculated that the PAS-evoked ACh release in sensorimotor cortex is autoinhibited in the TAC but not rivastigmine condition, and this could explain why rivastigmine but not TAC leads to enhancement of PAS-induced LTP-like plasticity.

At low-to-moderate therapeutic dose, MPH increases predominantly the extracellular concentration of NE in the brain and only to a much lesser extent the concentration of DA (Kuczenski and Segal, 2001). MPH enhances LTP in rat hippocampus and this effect is mediated by β-adrenergic receptor activation (Dommett *et al*, 2008). MPH effects on neocortical LTP have never been examined, and the only study on NE modulation of neocortical LTP also demonstrated LTP enhancement via β-adrenergic receptor activation in rat visual cortex (Bröcher *et al*, 1992). Given the absence of any data in M1 to compare with, the reasons for the lacking effect of MPH on PAS-induced LTP-like plasticity in this study remain unclear. It is unlikely that the MPH dose was inappropriate because in previous studies the same dose resulted in significant change in motor cortical inhibition and facilitation (Ilic *et al*, 2003) and in enhancement of motor practice-dependent plasticity (Meintzschel and Ziemann, 2006). Clearly, further studies are needed to resolve the question of to which extent it is at all possible to enhance LTP in M1 by agonists in the NE system.

Another possible explanation for the absence of enhancing effects by the agonists CAB, TAC, and MPH is saturation of PAS-induced LTP-like plasticity in the PBO condition because all included subjects had been screened for a significant LTP-like response (see Subjects and Methods). As a consequence, the LTP-like increase in MEP amplitude of 1.71 ± 0.05 in the PBO condition is one of the largest reported in the literature (Wolters *et al*, 2003; Stefan *et al*, 2004; Ziemann *et al*, 2004; Nitsche *et al*, 2007). Therefore, one might argue that LTP-like plasticity was saturated already under PBO conditions and could not be enhanced any further. However, the amount of LTP-like plasticity under PBO conditions is not critical because it is the (unknown) individual synaptic modification range of the corticospinal system that matters. Although we cannot fully rule the possibility that saturation of LTP-like plasticity has occurred in the present experiments, this is unlikely for the following two reasons: (1) unpublished experiments of our group demonstrate that it is possible to build up LTP-like plasticity significantly beyond a factor of 1.7 by a second PAS_{LTP} protocol if it follows the first PAS_{LTP} protocol by a delay of ~30 min (Müller-Dahlhaus *et al*, unpublished data); and (2) in the present study, 7/8 subjects had at least one value of PAS-induced LTP-like plasticity in one of the drug conditions exceeding the one in the PBO condition, and this 'maximum LTP-like plasticity' (1.96 ± 0.07) was significantly larger than LTP-like plasticity in the PBO condition ($P = 0.04$, two-tailed paired *t*-test).

Still, the selection of 'PAS responders' and the relatively small sample size constitute limitations of this study, and it is possible that inclusion of subjects lacking a PAS-induced LTP-like response might have revealed enhancement of LTP-like plasticity by CAB, MPH, or TAC.

The following paragraph provides possible explanations for the observed suppressive effects of HAL, PRZ, and BIP on PAS-induced LTP-like plasticity. Given that the selective dopamine D2 receptor antagonist sulpiride slightly (non-significantly) increased LTP-like plasticity (Nitsche *et al*, 2009), the clearly suppressive effect of HAL can only be understood by taking into account important differences between HAL and sulpiride. The most parsimonious reason is the lower affinity of sulpiride *vs* HAL at the dopamine D2 receptor (Matsubara *et al*, 1993). In addition, HAL inhibits the NMDAR containing NR1/2B subunits (Ilyin *et al*, 1996; Shim *et al*, 1999) but not the NMDAR containing NR1/2A. PAS-induced LTP-like plasticity is NMDAR dependent because it can be blocked by the noncompetitive NMDAR antagonist dextromethorphan (Stefan *et al*, 2002). Furthermore, NR1/2B rather than NR1/2A subunits containing NMDAR favor induction of LTP (Philpot *et al*, 2001). Another distinguishing feature is that HAL but not sulpiride has binding affinity to and blocks cortical á1-adrenergic receptors (Cohen and Lipinski, 1986; Patel *et al*, 2001). It is possible that blockade of á1-adrenergic receptors by HAL significantly contributed to its suppressive effect on PAS-induced LTP-like plasticity as we demonstrated a similar suppressive effect by PRZ (cf., Figure 3), a selective antagonist of the α1-adrenergic receptor. This idea is supported by a linear regression analysis, which revealed a highly significant correlation between the suppressions of PAS-induced LTP-like plasticity (expressed as difference of $MEP_{P1-P6/W2}$ in the drug minus PBO conditions) caused by HAL *vs* PRZ ($r = 0.86$, $P = 0.007$). The molecular mechanisms involved in the suppression of LTP by α1-adrenergic receptor blockade are as of yet unknown.

The suppressive effect of BIP on PAS-induced LTP-like plasticity constitutes an independent effect because HAL does not bind to cortical muscarinic receptors (Richelson and Souder, 2000). BIP is a selective antagonist at the muscarinic M1 receptor (Bolden *et al*, 1992). Although the role of muscarinic M1 receptors in motor cortical LTP has not been investigated, enhanced muscarinic M1 neurotransmission facilitates several forms of NMDAR-dependent hippocampal and corticostriatal LTP, whereas blockade of muscarinic M1 receptors suppresses these forms of LTP (Calabresi *et al*, 1999; Ovsepian *et al*, 2004). The most likely mechanism for this modulation is colocalization of muscarinic M1 receptors with NMDAR and potentiation of NMDAR currents by muscarinic M1 receptor activation (Marino *et al*, 1998).

In summary, our data suggest that LTP-like plasticity in human motor cortex is easily suppressed by antagonists of major neuromodulatory neurotransmitter systems, whereas enhancement of LTP-like plasticity is more difficult to obtain. This is in line with experiments in preparations of rat neocortex demonstrating that in contrast to LTP induction in primary somatosensory cortex, LTP induction in M1 does not show postsynaptic potential facilitation during repetitive burst stimulation in the LTP induction phase, and stable LTP can be obtained only under conditions of local disinhibition (Castro-Alamancos *et al*, 1995).

Clinical Perspective

The present findings bear on LTP-dependent processes such as motor learning in healthy subjects and motor re-learning in patients after central lesions. DA, NE, and ACh antagonists degrade practice-dependent plasticity in healthy subjects (Sawaki *et al*, 2002, 2003; Meintzschel and Ziemann, 2006), and retrospective studies strongly suggest that these NMDs are also detrimental in sensorimotor recovery after cerebral stroke (Goldstein *et al*, 1990; Goldstein, 1995). Conversely, DA, NE, and ACh agonists facilitate practice-dependent plasticity in healthy subjects (Bütefisch *et al*, 2002; Flöel *et al*, 2005a; Meintzschel and Ziemann, 2006) and may be beneficial in stroke rehabilitation (Crisostomo *et al*, 1988; Walker-Batson *et al*, 1995; Grade *et al*, 1998; Scheidtmann *et al*, 2001; Berthier *et al*, 2003; Flöel *et al*, 2005b; Zittel *et al*, 2007) although this evidence is not undisputed (for review, see Rösser and Flöel, 2008; Berends *et al*, 2009). The congruence of suppressive effects of NMDs on PAS-induced LTP-like plasticity and practice-dependent plasticity suggests that PAS-induced LTP-like plasticity may serve as a biological marker for unfavorable drug effects on motor learning and recovery. On the other hand, the differences with respect to enhancing effects suggest that PAS-induced LTP-like plasticity and practice-dependent plasticity are overlapping but not identical processes.

Finally, the present data are also pertinent to pathological conditions. Impaired PAS-induced LTP-like plasticity is typically observed in disorders associated with a dysfunctional dopaminergic system such as Parkinson's disease (Morgante *et al*, 2006; Ueki *et al*, 2006; Schwingenschuh *et al*, 2010) or schizophrenia (Frantseva *et al*, 2008), or a deficient central cholinergic system such as Alzheimer's disease (Battaglia *et al*, 2007), whereas exaggerated PAS-induced LTP-like plasticity can be observed in states of increased endogenous central cholinergic tone such as dystonia (Quartarone *et al*, 2003, 2008; Weise *et al*, 2006; Schwingenschuh *et al*, 2010).

In conclusion, we have demonstrated that antagonists in major neuromodulatory neurotransmitter systems suppress LTP-like plasticity at the systems level of human cortex, in accord with evidence of their modulating action of LTP at the cellular level. This provides further supportive evidence for the known detrimental effects of these drugs on LTP-dependent mechanisms such as learning and memory.

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