Behavioral/Systems/Cognitive

Focusing Effect of Acetylcholine on Neuroplasticity in the Human Motor Cortex

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Cholinergic neuromodulation is pivotal for arousal, attention, and cognitive processes. Loss or dysregulation of cholinergic inputs leads to cognitive impairments like those manifested in Alzheimer's disease. Such dysfunction can be at least partially restored by an increase of acetylcholine (ACh). In animal studies, ACh selectively facilitates long-term excitability changes induced by feed-forward afferent input. Consequently, it has been hypothesized that ACh enhances the signal-to-noise ratio of input processing. However, the neurophysiological foundation for its ability to enhance cognition in humans is not well documented. In this study we explore the effects of rivastigmine, a cholinesterase inhibitor, on global and synapse-specific forms of cortical plasticity induced by transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS) on 10–12 healthy subjects, respectively. Rivastigmine essentially blocked the induction of the global excitability enhancement elicited by anodal tDCS and revealed a tendency to first reduce and then stabilize cathodal tDCS-induced inhibitory aftereffects. However, ACh enhanced the synapse-specific excitability enhancement produced by facilitatory PAS and consolidated the inhibitory PAS-induced excitability diminution. These findings are in line with a cholinergic focusing effect that optimizes the detection of relevant signals during information processing in humans.

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

Introduction

Extensive evidence concerning cholinergic modulation of several cognitive functions supports an important role of acetylcholine (ACh) in arousal, attention, learning, and memory formation (Gold, 2003; Sarter et al., 2003). In Alzheimer's disease, enhancing cerebral ACh level has been shown to improve impaired learning and memory functions caused by cholinergic dysfunction. With regard to its specific functional properties, neurophysiological data from animal studies reveal dual neuromodulatory effects of ACh on cortical excitability and synaptic plasticity (Rasmusson, 2000; Gu, 2002). Cholinergic blockade has been shown to reduce long-term potentiation (LTP), whereas cholinergic agonists enhance LTP in the hippocampus, piriform cortex, and neocortex (Blitzer et al., 1990; Brocher et al., 1992; Hasselmo and Barkai, 1995). In humans, use-dependent plasticity of the motor cortex is facilitated by an acetylcholinesterase inhibitor and blocked by a cholinergic antagonist (Sawaki et al., 2002; Meintzschel and Ziemann, 2006). However, it is also reported that ACh enhanced long-term depression (LTD) induced with paired-pulse stimulation in the rat visual cortex (Kirkwood et al., 1999). Furthermore, it suppressed excitatory glutamatergic synaptic transmission via presynaptic inhibition at intrinsic, recurrent synapses but not afferent fiber synapses (Hasselmo and Bower, 1992; Hasselmo et al., 1995; Vogt and Regehr, 2001). This suggests a differential, activity-dependent cholinergic modification of neural networks in which ACh facilitates the detection of incoming afferent inputs, whereas it decreases intrinsic feedback excitability, thereby focusing the encoding of relevant, associated information processing (Blokland et al., 1992; Winters and Bussey, 2005).

To test the focusing action of ACh on neuroplasticity in humans, two protocols of brain stimulation were introduced in the present study. In the paired associative stimulation (PAS) protocol, repetitive peripheral nerve stimulation is paired with transcranial magnetic stimulation (TMS) of the human motor cortex (Stefan et al., 2000). It is postulated that PAS-induced excitability changes share the features of associative synaptic LTP and LTD, depending on the sequence of the near-synchronous pair of stimuli from different stimulation modalities in the motor cortex (Stefan et al., 2000; Wolters et al., 2003), which parallels the spike-timing-dependent rule for Hebbian LTP and LTD induction in animal studies (Dan and Poo, 2004). Facilitatory PAS (PAS25) with peripheral nerve stimulation applied 25 ms earlier than TMS pulse in M1 results in synchronous activation of motor cortical neurons by the afferent somatosensory stimulus and motor cortex TMS and thus enhances cortical excitability. However, inhibitory PAS (PAS10) diminishes cortical excitability with the interstimuli interval 10 ms, with which the somatosensory input reaches the motor cortex relevantly later than the TMS pulse, thereby inducing asynchronous stimulation on motor cortical neurons. PAS should specifically induce neuroplasticity in somatosensory-motor cortical synapses. In contrast, transcranial

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direct current stimulation (tDCS) encompasses the global modulation of cortical network plasticity by application of weak direct currents through the surface of the scalp. Anodal tDCS enhances cortical excitability, whereas cathodal tDCS diminishes it for up to 1 h after the end of stimulation (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2003a). The primary mechanism is a modulation of the resting membrane potential, and the resulting polarity-specific excitability changes subsequently induce changes of synaptic strength, which are, however, not restricted to specific synaptic connections (Bindman et al., 1964; Purpura and McMurtry, 1965). Both plasticity-inducing protocols accomplish long-lasting, NMDA receptor-dependent excitability changes (Liebetanz et al., 2002; Stefan et al., 2002; Nitsche et al., 2003b). The main difference lies in the synapse-specific focal effects of PAS: whereas the plasticity induced by tDCS is relatively nonfocal and not synapse-specific because it is thought to change cortical excitability under the whole area covered by the relatively large stimulation electrode, the plasticity induced by PAS is restricted to the intercortical connections between the somatosensory and motor cortex. According to the focusing hypothesis of ACh, it should selectively enhance and consolidate specific synaptic modifications induced by PAS, while depressing global ones accomplished by tDCS, to sharpen the signal-to-noise ratio in human cortical networks.

Materials and Methods

Subjects. Ten to twelve healthy subjects [tDCS experiment: six men, six women, aged (mean ± SD) 24 ± 3 years; PAS25 experiment: four women and six men, aged 28 ± 4 years; PAS10 experiment: 5 women and 5 men, aged 27 ± 4 years], without receiving acute or chronic medication, participated in each experiment. The groups did not differ significantly with regard to age and gender. Both studies were approved by the ethics committee of the University of Goettingen, and we conform to the Declaration of Helsinki. All subjects had given written informed consent. Because we were interested primarily in the physiological effects of ACh in this study, we recruited relatively young subjects to guarantee the comparability of the results with former pharmacological studies on neuroplasticity and to avoid including subjects with subclinical (e.g., microvascular) brain lesions, which might have influenced our results unintentionally.

Transcranial direct current stimulation. tDCS was performed with a pair of saline-soaked surface sponge electrodes (35 cm^2) with one of the electrodes placed over the representational area of the right abductor digiti minimi muscle (ADM), as determined by TMS, and the other electrode above the right orbit as reference. The currents ran continuously for 13 min (anodal tDCS) or 9 min (cathodal tDCS) with an intensity of 1 mA. In previous studies, these stimulation durations have been shown to induce aftereffects of tDCS lasting for ~1 h (Nitsche and Paulus, 2001; Nitsche et al., 2003a).

Paired associative stimulation. Peripheral nerve stimulation was applied on the right ulnar nerve at the level of the wrist with a Digitimer D185 stimulator (Digitimer, Welwyn Garden City, UK). Single-pulse TMS was delivered over the representing area of the right ADM. Each TMS pulse, at an intensity eliciting a muscle evoked potential of ~1 mV peak-to-peak amplitude, was preceded by an ulnar nerve stimulus with an interval of 25 ms (PAS25) or 10 ms (PAS10) using a standard stimulation block (cathodal proximal) at a stimulation width of 200 μs and stimulation intensity of 300% of the perceptual threshold, defined as the lowest intensity of the stimuli that is perceivable by the subject. Ninety pairs were applied at 0.05 Hz over 30 min, which has been shown to induce long-lasting excitability changes in the motor cortex (Stefan et al., 2000).

Pharmacological interventions. Rivastigmine (3 mg) or equivalent placebo drugs were taken by the subjects 2 h before the start of the intervention (Kennedy et al., 1999). This dose was chosen to minimize drug-induced side effects, but to enhance the cholinergic level of the CNS effectively. The experimental sessions were performed in a randomized order and were separated by at least 1 week to avoid cumulative drug or stimulation effects.

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

Experimental procedures. The experiments were conducted in a repeated measurement design. For the tDCS experiment, a complete crossover design was chosen. For the PAS experiments, separate subject groups participated in the PAS10 and PAS25 experiments. Subjects were seated comfortably in a reclining chair. First the optimal position of the magnetic coil for eliciting MEPs in the resting ADM was assessed over the left motor cortex, and 20 MEPs were recorded for the first baseline. Two hours after intake of the medication, a second baseline was determined to control for a possible influence of the drug on cortical excitability and adjusted if necessary. Subjects were not aware about and could not distinguish between the specific stimulation protocols used, nor were they informed about the medication administered in a specific session.

In experiment 1 with tDCS, one of the DC electrodes, to which in the following the terms cathodal or anodal tDCS refer, was fixed at the cortical representational area of ADM as defined during the first baseline recording, and the other one was fixed at the contralateral forehead area above the right orbit. Direct currents were applied on 12 subjects for 9 min (cathodal) or 13 min (anodal). After cessation of tDCS, 20 MEPs were recorded at 0.25 Hz every 5 min for half an hour and then every 30 min until 2 h after the end of DC stimulation, because tDCS-induced aftereffects without medication will not last longer than this period of time (Nitsche and Paulus, 2000; Nitsche et al., 2003a). Because of the relatively diurnal stability of corticospinal excitability, we did not expect additional excitability changes after MEPs returned to baseline level again. Only for the rivastigmine conditions, TMS recordings were performed at four additional time points: same day evening, next morning, next noon, and next evening.

In experiment 2 with PAS, the interventional PAS protocol as described above was used on 10 subjects. TMS recording procedures were the same as described above.

Data analysis and statistics. MEP amplitude means were calculated first individually and then interindividually for each time bin including both baseline values. The postintervention MEPs were normalized and are given as ratios of the baseline determined immediately before intervention (tDCS/PAS).

In the tDCS experiment, a repeated-measure ANOVA for the time bins up to 120 min after tDCS was calculated with the within-subject factors time course, current stimulation (anodal and cathodal tDCS), drug condition (rivastigmine vs placebo), and the dependent variable MEP amplitude. For PAS experiment, we performed a repeated-measure ANOVA with PAS (PAS25 and PAS10) as between-subject factor and within-subject factors drug condition (rivastigmine vs placebo) and time course for MEPs up to 120 min after intervention. If appropriate, *post hoc* Student's *t* tests (paired samples, two-tailed, $p < 0.05$, not adjusted) were performed to determine whether the MEP amplitudes before and after the interventional brain stimulations differed in each intervention condition and whether those differences depended on the drug conditions.

Table 1. Results of the ANOVAs

	Parameters	df	*F* value	*p* value
Experiment 1 (tDCS)	TDCS	1	40.585	< 0.001*
	Drug	1	3.650	0.082
	Time course	10	1.700	0.099
	tDCS × drug	1	3.417	0.092
	tDCS × time course	10	1.868	0.066
	Drug × time course	10	0.290	0.976
	tDCS × drug × time course	10	4.206	< 0.001*
Experiment 2 (PAS)	PAS	1	73.006	< 0.001*
	Drug	1	0.140	0.712
	Time course	10	1.556	0.123
	Drug × PAS	1	23.886	< 0.001*
	Time course × PAS	10	9.934	< 0.001*
	Drug × time course	10	2.207	0.019*
	Drug × time course × PAS	10	3.208	0.001*

In both experiments, the ANOVAs encompass the time course up to 120 min after tDCS or PAS, because the remaining time points were only measured for the rivastigmine conditions. "Drug" represents rivastigmine and placebo, whereas "tDCS" indicates anodal and cathodal polarity, and "PAS" represents PAS25 and PAS10. $p \leq 0.05$.

Additional *post hoc* tests (Student's *t* tests, $p < 0.05$) were performed to explore whether rivastigmine modified baseline MEPs.

Results

Three subjects experienced prominent side effects under rivastigmine (nausea, vomiting, and dizziness) and were excluded from the experiment. Two of the participating subjects complained about slight nausea after taking rivastigmine, but the symptoms subsided before the neuroplasticity-inducing intervention. Baseline MEP amplitudes did not differ significantly before and after drug intake in all conditions. Absolute baseline MEP amplitudes were not different in all medication and stimulation subgroups (Student's *t* tests, paired, two-tailed, $p > 0.05$).

Effects of rivastigmine on tDCS-induced motor cortex excitability shifts (experiment 1)

The ANOVA revealed a significant main effect of tDCS ($F = 40.585$; $p < 0.001$) and tDCS × drug × time course ($F = 4.206$; $p < 0.001$) (Table 1). In the PLC conditions, the anodal tDCS-induced excitability increase stayed significant until 30 min after tDCS, and the cathodal tDCS-induced inhibition lasted until 60 min after DC stimulation. As revealed by the *post hoc t* tests (paired, two-tailed, $p < 0.05$), rivastigmine initially abolished the induction of both the anodal tDCS-elicited excitability enhancement and the cathodal tDCS-elicited excitability diminution. However, a delayed, consolidated inhibition induced by cathodal tDCS was observed in the rivastigmine condition, compared with placebo conditions. The decrease of excitability generated by cathodal tDCS under rivastigmine remained significant for 2 h after tDCS, whereas it returned to baseline level after 60 min in the PLC condition (Fig. 1).

Effects of rivastigmine on PAS-induced motor cortex excitability shifts (experiment 2)

The ANOVA displayed a significant main effect of PAS ($F = 73.006$; $p < 0.001$). The interactions between drug × PAS ($F = 23.886$; $p < 0.001$), time course × PAS ($F = 9.934$; $p < 0.001$), drug × time course ($F = 2.207$; p 0.019), and drug × time course × PAS ($F = 3.208$; $p < 0.001$) were also significant (Table 1). In the PAS25 experiment, the excitatory shift of MEP amplitudes returned to baseline 25 min after PAS in the placebo medication condition, as revealed by Student's *t* tests (paired, two-tailed, $p < 0.05$), whereas rivastigmine enhanced and prolonged the excitatory effects of PAS25 (Fig. 2). In the PAS10 experiment, the inhibitory effect without medication lasted for half an hour. Rivastigmine further enhanced and prolonged the inhibition until the same day evening after intervention (Fig. 3).

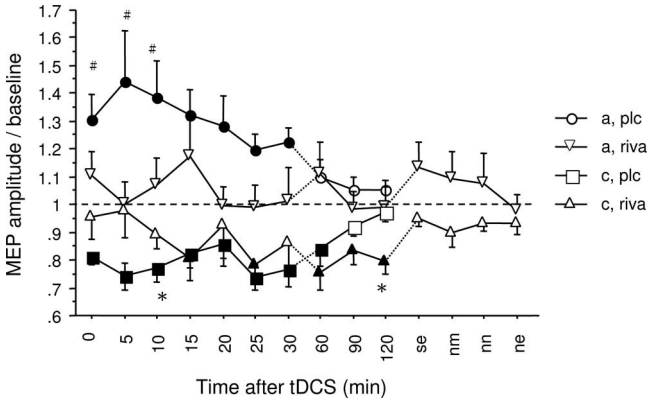

Figure 1. Cholinergic modulation of global cortical plasticity induced by tDCS. Rivastigmine abolished the induction of anodal tDCS-elicited excitability increases, as recorded by TMS-evoked MEP amplitudes. Additionally, rivastigmine initially diminished the excitability reduction induced by cathodal tDCS under rivastigmine. However, the respective excitability diminution was later consolidated. Filled symbols indicate significant deviations from baseline with regard to each drug condition. Hash symbols indicate significant differences in anodal tDCS-induced excitability changes between placebo and rivastigmine conditions; asterisks represent significant differences in inhibition caused by cathodal stimulation between the placebo and rivastigmine medication conditions (Student's *t* test, two-tailed, repeated measures; $^{\#}p < 0.05$). a, Anodal; c, cathodal; plc, placebo; riva, rivastigmine; se, same evening; nm, next morning; nn, next noon; ne, next evening. Error bars indicate SEM.

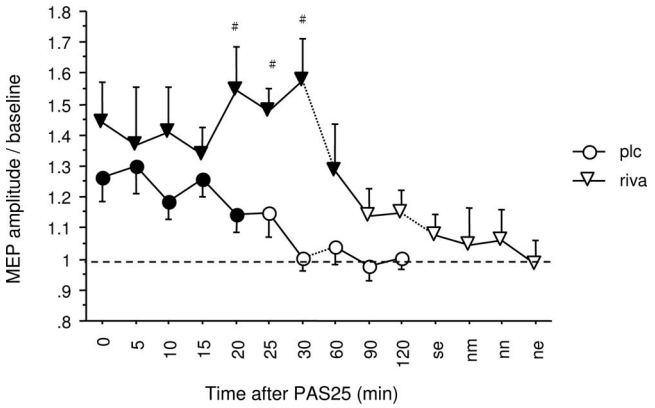

Figure 2. The PAS25-induced synapse-specific excitability enhancement is facilitated by ACh. The PAS-induced excitability enhancement was increased and prolonged under rivastigmine until 30 min after PAS, whereas MEP amplitudes returned to baseline 25 min after PAS in the placebo condition. Hash symbols represent significant differences between placebo and rivastigmine conditions; filled symbols indicate significant deviations from baseline with regard to each drug condition (Student's *t* test, two-tailed, repeated measures; $^{\#}p < 0.05$). plc, Placebo; riva, rivastigmine; se, same evening; nm, next morning; nn, next noon; ne, next evening. Error bars indicate SEM.

Discussion

The present study demonstrates that ACh enhances the synapse-specific cortical excitability increase induced by PAS25 and consolidates the PAS10-induced reduction of motor cortical excitability, whereas it prevents global excitatory aftereffects produced by anodal tDCS. ACh also delayed the induction of the cathodal tDCS-elicited excitability decrease and slightly prolonged its overall duration. Because MEP amplitudes were not modified by rivastigmine in the dosage applied alone, we have no evidence for a direct cholinergic influence of the drug on corticospinal excit-

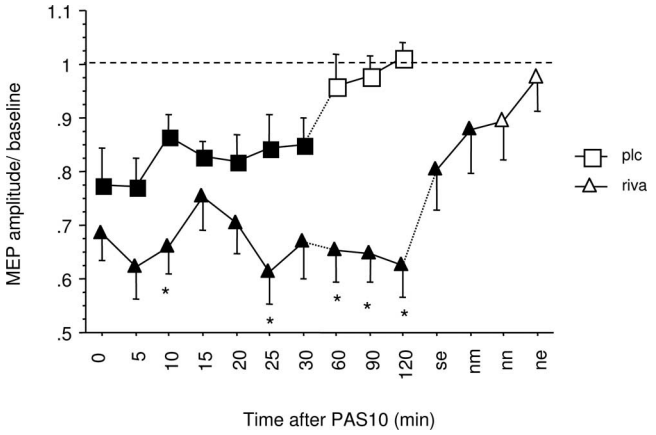

Figure 3. The PAS10-induced synapse-specific excitability diminution is consolidated by ACh. In the placebo condition, the inhibitory effect of PAS10 lasted for approximately half an hour, whereas rivastigmine enhanced the inhibition and further prolonged it until same day evening of the intervention. Significant differences between placebo and rivastigmine are shown with asterisks. Filled symbols indicate significant deviations from baseline with regard to each drug condition (Student's *t* test, two-tailed, repeated measures, $p < 0.05$). plc, Placebo; riva, rivastigmine; se, same evening; nm, next morning; nn, next noon; ne, next evening. Error bars indicate SEM.

ability. The results support the hypothesis of a focusing effect of ACh on neuroplasticity of cortical networks. ACh not only increased selectively the efficacy of synapse-specific excitability-enhancing neuroplasticity, but also prolonged the excitability diminution in case of asynchronous synapse-specific inputs and suppressed global excitability enhancements. By these mechanisms, ACh is well suited to improve the signal-to-noise ratio and to refine information processing in neural networks.

ACh diminishes tDCS-driven neuroplasticity

At first glance, the inhibitory effect of rivastigmine on facilitatory neuroplasticity induced by anodal tDCS seems contradictory to the results obtained from animal studies in which LTP was facilitated by cholinergic stimulation (Brocher et al., 1992; Abe et al., 1994; Hasselmo and Barkai, 1995; Patil et al., 1998). The major conceptual difference between these studies and our tDCS experiment is the manipulation applied for neuroplasticity induction. In slice preparation, the plastic changes induced with either paired or high-frequency suprathreshold electrical stimulation are caused by synchronous activation of synaptic connections. In contrast, tDCS-elicited neuroplasticity is a consequence of increasing general, asynchronous network activity, possibly added by modification of the postsynaptic resting membrane potential (Bindman et al., 1964; Purpura and McMurtry, 1965). The diminution of the anodal tDCS-induced excitability enhancement might thus be because of an ACh-induced decrease of general excitation within global neuronal networks, probably modulated via the cholinergic presynaptic inhibition of excitatory feedback potentials or excitatory transmission at recurrent connections. Moreover, evidence reveals that the suppression of synaptic transmission is selective for recently modified synapses (Linster et al., 2003) but does not apply to silent synapses (Fernandez de Sevilla et al., 2002). Thus it is probable that synapses that are globally modified by tDCS in the present study are more susceptible to cholinergic suppression of synaptic transmission during plasticity induction. A similar effect is also demonstrated by a recent study using a dynamic clamp system to mimic *in-vivo*-like background activities in motor cortical slices, in which cholinergic facilitation of LTP was attenuated in the presence of random background noise (Desai and Walcott, 2006).

However, ACh revealed a tendency to consolidate excitability-diminishing aftereffects generated by cathodal tDCS, although it abolished its initial induction phase. The biphasic effect of rivastigmine on cathodal tDCS-induced plasticity can also be explained by a cholinergic regulation of different neurons within different temporary profiles. The initial blockade could be because of the fast negative modulation of inhibitory neurons (e.g., inhibitory interneurons) known to be induced by ACh (Ji and Dani, 2000), whereas the late-onset and prolonged excitability diminution can be explained by the inhibitory modification of excitatory neurons with the combination of ACh and cathodal tDCS. Further studies are required to test this hypothesis.

Cholinergic consolidation of PAS-induced cortical plasticity

The PAS experiment demonstrates a positive cholinergic modulation of PAS-elicited synaptic-specific plasticity. ACh seems to enhance specifically the ability of synchronous motor cortical input to enhance excitability, whereas the excitability diminution accomplished by asynchronous input is prolonged. It is suggested that PAS25/PAS10 relates to associative LTP/LTD in the human motor cortex (Stefan et al., 2000, 2002; Wolters et al., 2003; Stefan et al., 2006). Because ACh has been shown to facilitate cortical sensory plasticity by enhancing sensory input processing (Rasmusson and Dykes, 1988; Tremblay et al., 1990; Patil et al., 1998) and suppressing irrelevant input (Hasselmo and Barkai, 1995), one might further speculate that rivastigmine specifically improved the efficacy of PAS by (1) enhancing the signal-to-noise ratio, thereby facilitating meaningful information processing as represented by synchronous input; and (2) suppressing non-meaningful input as represented by asynchronous stimulation within neural networks.

Thus, the results of our study are in accordance with the respective animal experiments (Blitzer et al., 1990; Hasselmo and Barkai, 1995; Kirkwood et al., 1999). Moreover, they are concordant with the results of recent behavioral studies in the human motor cortex exploring the effect of ACh modulation on use-dependent plasticity, which was blocked by the ACh antagonist scopolamine (Sawaki et al., 2002) and enhanced by an acetylcholinesterase inhibitor (Meintzschel and Ziemann, 2006). Therefore, our results offer a neurophysiological mechanism of how ACh might improve behavioral plasticity.

It is also notable that the duration of cholinergic effects on cortical plasticity induced by PAS25 and PAS10 is asymmetric. ACh was more effective in stabilizing the PAS10-generated excitability reduction than the PAS25-induced excitability enhancement. This might be because of the generally less efficient PAS25 protocol, as revealed in the placebo condition in the present study, which might have limited the ability of ACh to stabilize neuroplasticity. Alternatively, it was suggested that the direction of cholinergic modulation on synaptic plasticity could be determined by ACh concentration and subtype receptors activation (Kuczewski et al., 2005), which could also explain an asymmetry of ACh effects on different PAS protocols.

Summary of cholinergic modulation in human cortical plasticity

The results of the present study suggest that ACh has fairly specific effects on cortical plasticity. Rivastigmine selectively enhanced the efficacy of PAS25, a synchronous associative stimulation protocol, to increase excitability, whereas it shifted the effects of the remaining plasticity-inducing protocols in an inhib-

itory direction. This indicates a general inhibitory effect of rivastigmine on network excitability, with the exception of PAS25-induced plasticity, and further explains how rivastigmine works as a cognitive enhancer via increasing the signal-to-noise ratio of cortical activity. Associative plasticity is suggested as a neurophysiological correlate of learning and memory formation, and, indeed, excitability-enhancing PAS has been shown to be tightly connected to learning processes (Ziemann et al., 2004; Stefan et al., 2006), thus strengthening the efficacy of synchronous stimuli to enhance excitability might improve learning. However, rivastigmine can reduce "noisy" synaptic modification, as demonstrated in our study via abolishing the excitability-enhancing properties of anodal tDCS, which increases general and most probably not synchronous cortical activity (Bindman et al., 1964; Purpura and McMurtry, 1965), and via strengthening the inhibitory effects of the asynchronous PAS10 protocol. Moreover, rivastigmine enhanced the efficacy of cathodal tDCS to diminish cortical excitability. Cathodal tDCS applied synchronously with PAS25 has been shown to enhance the efficacy of PAS25 to increase cortical excitability (Nitsche et al., 2007), which can be explained by a noise-reducing function of cathodal tDCS-induced inhibition. Thus the enhancing effect of rivastigmine on cathodal tDCS-driven inhibition should also increase the efficacy of meaningful information processing via increasing the signal-to-noise ratio.

A recently conducted study revealed similar effects of L-dopa (L-3,4-dihydroxyphenylalanine) on neuroplasticity (Kuo et al., 2007). Here, dopamine reversed the anodal tDCS-induced excitability enhancement into inhibition and prolonged the excitability diminution caused by cathodal tDCS. However, dopamine enhanced the PAS25-induced synapse-specific increase of cortical excitability. The similarity of the dopamine and ACh effects could be explained by the fact that dopaminergic and cholinergic neurons serve similar functions and are tightly interconnected in neuronal networks (Sarter et al., 1999; Zahm, 2006). They also provide, at least, a theoretical option for the application of dopamine in neurological disorders associated with cognitive deficits. Indeed, dopaminergic medication has been shown to improve learning in healthy subjects and patients after stroke (Floel et al., 2005, 2007).

Together, the results of the present study are in line with cortical cholinergic functions enhancing the contrast of relevant stimuli against background noise or distractors, thereby improving signal processing during information encoding. Therefore, it might strengthen the rational basis for application of cholinesterase inhibitors to improve cognitive functions in patients with Alzheimer's disease or vascular dementia. However, it has to be kept in mind that the results were obtained with healthy young subjects. Further studies are needed to elucidate whether the effect of ACh on neuroplasticity is identical in elderly healthy and demented patients and whether the impact of ACh on neuroplasticity is correlated with clinical outcome.

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