Nicotine alters cognitive functions in animals and humans most likely by modification of brain plasticity. In the human brain, it alters plasticity induced by transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS), probably by interference with calcium-dependent modulation of the glutamatergic system. We aimed to test this hypothesis further by exploring the impact of the α4β2-nicotinic receptor partial agonist varenicline on focal and non-focal plasticity, induced by PAS and tDCS, respectively. We administered low (0.1 mg), medium (0.3 mg), and high (1.0 mg) single doses of varenicline or placebo medication before PAS or tDCS on the left motor cortex of 25 healthy non-smokers. Corticospinal excitability was monitored by single-pulse transcranial magnetic stimulation-induced motor evoked potential amplitudes up to 36 h after plasticity induction. Whereas low-dose varenicline had no impact on stimulation-induced neuroplasticity, medium-dose abolished tDCS-induced facilitatory after-effects, favoring focal excitatory plasticity. High-dose application preserved cathodal tDCS-induced excitability diminution and focal excitatory PAS-induced facilitatory plasticity. These results are comparable to the impact of nicotine receptor activation and might help to further explain the involvement of specific receptor subtypes in the nicotinic impact on neuroplasticity and cognitive functions in healthy subjects and patients with neuropsychiatric diseases.

Keywords: human motor cortex, neuroplasticity, nicotine, paired associative stimulation, transcranial direct current stimulation

Introduction

Smoking tobacco is one of the leading risks to human health (Peto et al. 1992; Doll et al. 2005). Nicotine is the main neuroactive component of tobacco responsible for physical dependence and addiction. Besides addictive properties, many studies demonstrate positive effects on cognition. Human and animal studies have shown that nicotine improves attention, motor functions, working, and episodic memories (Provost and Woodward 1991; Hahn and Stolerman 2002; Hahn et al. 2002; Kumari et al. 2003; Jubelt et al. 2008; Froeliger et al. 2009; Heishman et al. 2010; Mocking et al. 2012). Nicotine also improves learning, attention, and perception in patients suffering from Alzheimer’s disease (Jones et al. 1992; Wilson et al. 1995; White and Levin 1999). Nicotine withdrawal is often associated with impairments of working and verbal memory and neuroplasticity, while nicotine re-administration reinstates these functions in smoking individuals (Jacobsen et al. 2005; Cole et al. 2010; Grundey et al. 2012a, 2012b).

The neurophysiological basis for the nicotinic effects on cognition is hypothesized to be its impact on cortical excitability and plasticity, controlled by activation of α4β2 and α7 nicotinic acetylcholine receptors (nAChRs). These are ligand-gated ion channels (Burnashev 1998; Dajas-Bailador and Wonnacott 2004), which modulate the permeability of Ca2+ ions and are centrally involved in plasticity induction (Lisman 2001). In accordance, animal studies have demonstrated that activation of nicotinic receptors results in long-term potentiation (LTP) facilitation (Matsuyama et al. 2000; Fujii and Sumikawa 2001a, 2001b; Welsby et al. 2006; Nakauchi et al. 2007), reversal of GABAergic inhibition of LTP (Fujii et al. 2000), as well as long-term depression (LTD) enhancement (Fujii and Sumikawa 2001a, 2001b; Ge and Dani 2005).

Recently, studies in humans demonstrated that global cholinergic activation increases focally, but abolishes non-focal induced LTP-like plasticity, whereas it preserves and prolongs both focal and non-focal LTD-like plasticity. For nicotine, a similar effect was seen for LTP-like plasticity, but LTD-like plasticity was abolished by this substance in non-smoking healthy humans (Kuo et al. 2007; Thirugnanasambandam et al. 2012). These results show a partial dissociation of the impact of global cholinergic activation and nicotinic receptor activation on plasticity. Furthermore, the “focusing effect” on LTP-like plasticity might explain a beneficial impact on cognition.

In these studies, focal and non-focal plasticity were induced by transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS), respectively. Both tDCS and PAS are non-invasive brain stimulation techniques inducing lasting changes of cortical excitability, which are Ca2+ and NMDA receptor-dependent (Nitsche and Paulus 2000, 2001; Stefan et al. 2000, 2002; Nitsche, Fricke, et al. 2003; Nitsche et al. 2008). Neuroplastic changes induced by DCS are non-focal and affect neuronal populations beneath the relatively large stimulation electrodes via subthreshold resting membrane potential modulation (Purpura and McMurtry 1965; Nitsche, Roth, et al. 2007; Nitsche et al. 2008). In contrast, plasticity induced by PAS is presumed to be focal, synapsec-specific, and timing-dependent, affecting only selective neuronal populations. During PAS, a repetitive electric pulse to a peripheral nerve is combined with a suprathreshold transcranial magnetic stimulation (TMS) pulse over the corresponding area of the primary motor cortex. The target group of somatosensory–motor cortical synaptic connections is activated synchronously or asynchronously by combined peripheral and TMS pulses, depending on the interstimulus interval (ISI), resulting in excitatory or inhibitory after-effects (Stefan et al. 2000). PAS is thought to be closely linked to learning and memory processes, as its mechanism resembles some characteristics of spike-timing-dependent plasticity (Stefan et al. 2002; Wolters et al. 2003; Caporale and Dan 2008).

Beyond unspecified activation of nicotinic receptors by nicotine, not much is known about the contribution of nicotinic...
receptor subtypes on neuroplasticity in humans. Given that tDCSs and PAS induce calcium-dependent plasticity, it can be speculated that specifically nicotinic receptors with calcium channel properties might be involved. In accordance, in the present study, we aimed to explore the contribution of $\alpha_4\beta_2$ receptors on non-invasive brain stimulation-induced focal and non-focal plasticity in human non-smokers via application of varenicline. Varenicline is an effective smoking cessation agent (Coe et al. 2005), which is a high-affinity partial agonist to $\alpha_4\beta_2$ and full agonist to $\alpha_2$ nAChRs (Mihalak et al. 2006). Varenicline is also suggested to have therapeutic effects in patients suffering from Alzheimer’s disease (Kem 2000; Jensen et al. 2005), schizophrenia, depression during smoking abstinence (Hong et al. 2011; Liu et al. 2011; Shim et al. 2012; Anthenelli et al. 2013), and patients with ataxia (Zesiewicz et al. 2012). Studying the impact of varenicline on plasticity might thus not only be suited to explore the physiology of nicotinic receptor activation to a larger extent, but also help to comprehend its impact on clinical symptoms. We hypothesized that effective dosages of the drug should, similar to the effect of nicotine (Thirugnanasambandam et al. 2012), abolish tDCS-induced non-focal plasticity and preserve PAS-induced focal excitatory plasticity in non-smoking healthy subjects.

Materials and Methods

Subjects

Twenty-five healthy non-smokers aged 24.8±4.4 years (11 males/15 females) were recruited. Two subjects did not finish the experiment. One subject took part in both, the tDCS and PAS parts of the study. All subjects were right-handed according to the Edinburgh handedness inventory (Oldfield 1971). None of them took any medication, had a history of a neuropsychiatric or medical disease, present pregnancy, or metallic head implants. All volunteers gave written informed consent and were compensated for participation. The investigation was approved by the Ethics Committee of the University of Göttingen and conforms to the principles laid down in the Declaration of Helsinki.

Transcranial Direct Current Stimulation

Twelve subjects aged 24.4±4.7 years (4 males/8 females) participated in the tDCS experiment. Direct current was delivered by a battery-driven constant current stimulator (neuroConn GmbH, Ilmenau, Germany) through a pair of rubber electrodes covered with saline-soaked sponges (5×7 cm). The motor cortex electrode was fixed over the area representing the right abductor digitii minimi muscle (ADM) and the return electrode contralaterally above the right supraorbital area. Subjects received 1 mA of either excitability-enhancing anodal tDCS for 13 min or excitability-diminishing cathodal tDCS for 9 min, which induces motor cortex excitability alterations lasting for about 1 h (Nitsche and Paulus 2001; Nitsche, Nitsche, et al. 2003). To achieve a functionally monophasic stimulation over the primary motor cortex, an enlarged return electrode could have been used (Nitsche, Doemkes, et al. 2007). We did not use such a large return electrode to keep the experimental design identical to those of former studies of our group (Kuo et al. 2008; Monte-Silva et al. 2009, 2010; Thirugnanasambandam et al. 2012), since the size of the return electrode seems to have no impact on resulting motor cortex plasticity (Nitsche, Doemkes, et al. 2007).

Paired Associative Stimulation

Twelve subjects 25±4.4 years (6 males/6 females) participated in the PAS experiment. The peripheral electric pulse used over the right ulnar nerve at the level of the wrist at an intensity of 300% of the sensory perceptual threshold was followed by a TMS pulse over the M1 representation of the ADM at ISIs of 10 ms (PAS10) or 25 ms (PAS25) at a frequency of 0.05 Hz. The peripheral electric pulse was delivered by a Digitimer D184 multipulse stimulator (Digitimer, Welwyn Garden City, UK). The TMS pulse was delivered by a Magstim 200 stimulator with an intensity to elicit single-pulse motor evoked potentials (MEPs) with peak-to-peak amplitudes of on average 1 mV. The participants were instructed to silently count the number of pulses they received at their wrist during the whole stimulation duration to guarantee sufficient attention to the procedure, which has been shown to be crucial to obtain the desired after-effects (Stefan et al. 2000, 2004).

Pharmacological Interventions

Low (0.1 mg), medium (0.3 mg), or high (1.0 mg) dosages of varenicline or 0.5 mg placebo were administered in form of 2-piece gelatin capsules (size 2, 18 mm length, 6.35 mm external diameter) 3 h before the start of the experimental session, allowing the verum drug to induce a maximum plasma level and to produce prominent effects in the central nervous system. One milligram of varenicline is a single oral dosage administered in smokers to support cessation of tobacco consumption (Faesel et al. 2006, 2010; Obach et al. 2006).

Monitoring of Motor Cortical Excitability

MEPs were recorded from the right ADM by single-pulse TMS over the corresponding left primary motor cortex, conducted by a Magstim 200 magnetic stimulator (Magstim, Whiteland, Dyfed, UK) with a figure-of-eight magnetic coil (diameter of one winding—70 mm; peak magnetic field—2.2 T). The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from the midline. The hotspot was defined as the optimal coil placement, where the TMS pulse resulted consistently in the largest MEPs of the contralateral ADM. Surface MEPs were recorded from the right ADM with Ag-AgCl electrodes in a belly-tendon montage. The signals were amplified, band-pass filtered (2 Hz–2 kHz, sampling rate, 5 kHz), digitized with a micro-1401 AD converter (Cambridge Electronic Design, Cambridge, UK), controlled by the Signal Software (Cambridge Electronic Design, v. 2.13), and stored for offline analysis.

Experimental Procedures

A unique sequence of experimental sessions was randomly generated individually for each subject, which did not match any previously generated one for other subjects. The participants were seated in a comfortable chair with head and arm rests. In the beginning, the hotspot was identified by TMS and then the stimulation intensity was adjusted to elicit single-pulse MEPs with peak-to-peak amplitudes of on average 1 mV. Then, 25 MEPs were recorded for the determination of baseline. After baseline recording, varenicline or placebo medication was administered. Three hours after intake of medication, a second baseline was recorded to monitor for a possible impact of the drug alone on cortical excitability (baseline 2), and TMS intensity was adjusted, if necessary (baseline 3). After that procedure, the respective plasticity induction protocol was administered (cathodal tDCS, anodal tDCS, PAS10 or PAS25) and 25 MEPs were recorded at time points of 0, 5, 10, 15, 20, 25, 30, 60, 90, and 120 min after tDCS. Further TMS measurements were conducted in the evening of the same day (SE), next morning, at approximately 9:00 AM (NM), next noon, at approximately 12:00 PM (NN), and next evening, at approximately 6:00 PM (NE) (Fig. 1). To keep the surface electromyography electrodes and TMS coil at the same place for later measurements, their positions were marked with a waterproof pen. The minimum period between 2 consecutive experimental sessions for a single subject was 7 days. Subjects were blinded for both, stimulation and medication conditions; the experimenter was blinded for the medication condition.

Analysis and Statistics

The experimenter was unblinded after finishing data collection and analysis. The individual means of 25 MEP amplitudes were calculated at each time point for every subject, and the post-tDCS mean MEP amplitudes were normalized to the respective mean baseline MEP amplitudes (quotient of post-stimulation MEPs vs. pre-stimulation values).
baseline 2 or, if TMS intensity had to be adjusted, baseline 3). Then, the grand averages for each time point were calculated. A repeated-measures ANOVA was performed on the above-mentioned data separately for tDCS and PAS experiments, using MEP amplitude as the dependent variable and medication, stimulation type and time course as within-subject factors. The Mauchly test of sphericity was performed, and the Greenhouse–Geisser correction applied when necessary. In case of significant results of the ANOVA, exploratory post hoc comparisons were performed using Student’s t-tests (paired samples, two-tailed, \( P < 0.05 \), not corrected for multiple comparisons) between the MEP amplitudes before and after intervention within one experimental condition and between the single time points (medication vs. placebo) within the same stimulation condition.

To compare main effects of different dosages of varenicline on plasticity, averaged MEPs for the first 30 min after stimulation were calculated for each subject per experimental session and normalized to baseline 2 (or baseline 3, if TMS intensity was adjusted). Then, these averaged MEP values for each dosage condition were compared with the respective placebo condition by Student’s t-tests (paired samples, two-tailed, \( P < 0.05 \), not corrected for multiple comparisons).

To exclude differences between baseline values of different conditions, and also between first, second, and third baseline values, the respective values were compared using Student’s t-tests (paired samples, two-tailed, \( P < 0.05 \), not corrected for multiple comparisons).

### Results

All subjects tolerated the procedure well. Only 5 of them reported slight dizziness, lasting for about 1 h after drug intake, which is a mild side effect of varenicline.

Two participants (1 from tDCS and 1 from PAS experiment) left the study after the first experimental day due to time constraints.

The average baseline MEP values did not significantly differ between groups as revealed by Student’s t-tests (paired samples, two-tailed, \( P > 0.05 \)). Varenicline alone did not have any impact on cortical excitability at any dosage, as revealed by Student’s t-tests between first, second, and third baseline values (paired samples, two-tailed, \( P > 0.05 \)) (Table 1).

### Effect of Varenicline on tDCS-Induced Plasticity

The ANOVA revealed significant main effects of Stimulation (\( F_1 = 117.900; P < 0.001 \)), Medication \( \times \) Stimulation (\( F_3 = 5.050; P = 0.005 \)), Stimulation \( \times \) Time (\( F_{14} = 10.013; P < 0.001 \)), and Medication \( \times \) Stimulation \( \times \) Time (\( F_{42} = 2.375; P < 0.001 \)) interactions (for details see Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>TMS Parameter</th>
<th>Medication Condition</th>
<th>Baseline 1</th>
<th>Baseline 2</th>
<th>Baseline 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathedral tDCS MEPl</td>
<td>0.1 mg</td>
<td>0.95 ± 0.07</td>
<td>0.95 ± 0.07</td>
<td>0.96 ± 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>1.00 ± 0.17</td>
<td>0.94 ± 0.17</td>
<td>0.96 ± 0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>0.93 ± 0.13</td>
<td>0.94 ± 0.12</td>
<td>0.96 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.94 ± 0.09</td>
<td>0.94 ± 0.09</td>
<td>0.95 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>%MSO</td>
<td>0.1 mg</td>
<td>53.87 ± 7.67</td>
<td>53.87 ± 7.67</td>
<td>53.76 ± 7.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>53.58 ± 7.25</td>
<td>53.58 ± 7.25</td>
<td>53.92 ± 7.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>50.74 ± 7.29</td>
<td>50.74 ± 7.29</td>
<td>50.00 ± 7.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>53.50 ± 7.18</td>
<td>53.50 ± 7.18</td>
<td>53.58 ± 7.24</td>
<td></td>
</tr>
<tr>
<td>Anodal tDCS MEPl</td>
<td>0.1 mg</td>
<td>0.96 ± 0.13</td>
<td>0.95 ± 0.17</td>
<td>0.93 ± 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>1.00 ± 0.25</td>
<td>0.95 ± 0.07</td>
<td>0.94 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>0.98 ± 0.19</td>
<td>1.01 ± 0.42</td>
<td>0.99 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.97 ± 0.13</td>
<td>0.97 ± 0.12</td>
<td>0.98 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>%MSO</td>
<td>0.1 mg</td>
<td>53.67 ± 6.81</td>
<td>53.67 ± 6.81</td>
<td>53.83 ± 6.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>52.83 ± 6.81</td>
<td>52.83 ± 6.81</td>
<td>52.67 ± 6.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>53.33 ± 7.48</td>
<td>53.33 ± 7.48</td>
<td>53.42 ± 7.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>53.25 ± 7.90</td>
<td>53.25 ± 7.90</td>
<td>53.33 ± 7.91</td>
<td></td>
</tr>
<tr>
<td>PAS10 MEPl</td>
<td>0.1 mg</td>
<td>0.96 ± 0.12</td>
<td>0.95 ± 0.29</td>
<td>1.01 ± 0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>1.00 ± 0.16</td>
<td>1.00 ± 0.16</td>
<td>1.00 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>0.97 ± 0.09</td>
<td>0.99 ± 0.07</td>
<td>0.99 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>%MSO</td>
<td>0.1 mg</td>
<td>51.00 ± 9.05</td>
<td>51.00 ± 9.05</td>
<td>51.33 ± 9.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>52.17 ± 9.33</td>
<td>52.17 ± 9.33</td>
<td>52.25 ± 9.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>50.83 ± 9.46</td>
<td>50.83 ± 9.46</td>
<td>50.83 ± 9.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>51.83 ± 9.23</td>
<td>51.83 ± 9.23</td>
<td>51.83 ± 9.23</td>
<td></td>
</tr>
<tr>
<td>PAS25 MEPl</td>
<td>0.1 mg</td>
<td>0.99 ± 0.12</td>
<td>0.99 ± 0.14</td>
<td>1.00 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>1.00 ± 0.17</td>
<td>1.00 ± 0.17</td>
<td>1.00 ± 0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>0.98 ± 0.10</td>
<td>1.04 ± 0.16</td>
<td>1.02 ± 0.10</td>
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</tr>
<tr>
<td>%MSO</td>
<td>0.1 mg</td>
<td>52.83 ± 8.28</td>
<td>52.83 ± 8.28</td>
<td>53.08 ± 8.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg</td>
<td>52.00 ± 9.72</td>
<td>52.00 ± 9.72</td>
<td>51.92 ± 9.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>52.75 ± 9.12</td>
<td>52.75 ± 9.12</td>
<td>52.75 ± 9.08</td>
<td></td>
</tr>
<tr>
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<td>52.58 ± 8.56</td>
<td>52.58 ± 8.56</td>
<td>52.58 ± 8.56</td>
<td></td>
</tr>
</tbody>
</table>

Note: Shown are the mean MEP amplitudes ± SD and stimulation intensity (percentage of maximum stimulator output, %MSO) mean ± SD of baselines 1, 2, and 3. The intensity of TMS was adjusted to elicit MEPs with peak-to-peak amplitude of approximately 1 mV (baseline 1). A second baseline (baseline 2) was recorded 3 h after varenicline or placebo intake to determine the impact of the drug on cortical excitability and adjusted if necessary (baseline 3). Student’s t-tests revealed no significant differences between conditions (\( P > 0.05 \)).

Post hoc Student’s t-tests show that, in the placebo and low-dose varenicline medication conditions, the latter being 10 times lower than a single oral dosage (1 mg) administered in smokers to support cessation of tobacco consumption (Faessel et al. 2010), MEPs were significantly enhanced for 60 min after anodal and reduced after cathodal tDCS when compared with respective baseline values. MEPs obtained under low-dose varenicline did not differ from those under placebo medication at any time point. Medium-dose varenicline abolished both...
Discussion

The results of this study show that activation of nicotinic α4β2 and, possibly, α7 receptors has specific and dosage-dependent effects on neuroplasticity in healthy human non-smoking individuals. Low-dosage varenicline did not affect plasticity. In contrast, a medium dose of the drug induced collapse of non-focal, but preserved focal, LTP-like plasticity. Under high dosages of the drug non-focal LTP-like and focal LTD-like plasticity were compromised, whereas non-focal and focal LTP-like effects remained preserved. Preference of focal excitatory plasticity obtained under medium-dosage varenicline is fairly identical to the one of a previous study, which explored the impact of nicotine on tDCS-induced plasticity (Kuo et al. 2007; Thirugnanasambandam et al. 2012). Therefore, we presume that the focusing effect of nicotine on facilitatory plasticity is at least partially caused by α4β2 receptors. As the MEP amplitudes alone were not affected by any dose of varenicline, a direct influence of the drug on cortical excitability can be ruled out.

Proposed Mechanisms of Action

After-effects of tDCS and PAS are NMDA receptor- and Ca2+-dependent (Stefan et al. 2002; Nitsche, Fricke, et al. 2003; Wollers et al. 2003). Since α4β2 and α7 nAChRs are ligand-gated ion channels (Burnashev 1998; Dajas-Bailador and Wonnacott 2004), they might affect LTP and LTD induction by an alteration of membrane permeability to Ca2+ ions (Lisman 2001). Indeed, in animal slice experiments, agonists of the respective receptors have a prominent impact on stimulation-induced plasticity. Nicotine has been shown to enhance LTP by postsynaptically activating α7 nicotinic receptors in the rat dentate gyrus (Welsby et al. 2006), and facilitates NMDA-dependent LTD induction (Yamazaki et al. 2005; Yamazaki, Jia, et al. 2006; Yamazaki, Jia, et al. 2006; Griguoli et al. 2013; Prestori et al. 2013). In another study, activation of both α4β2 and α7 nicotinic receptors was essential for LTP induction (Matsumoto et al. 2003). Since activation of nAChRs increased intracellular Ca2+ in several studies (Chavez-Noriega et al. 1997, 2000; Khiroug et al. 2003; Karadsheh et al. 2004; Fayuk and Yakel 2005, 2007; Jia et al. 2010), this effect is most probably accomplished by calcium concentration alterations.

At first glance, the impact of nicotinic receptor enhancement on plasticity in the present experiment is not completely compatible with the direction of effects obtained in the above-mentioned animal experiments, especially with regard to LTD induction. However, the key for understanding the results might be the non-linear impact of calcium on plasticity. Whereas low intraneuronal calcium enhancement induces LTD, high concentrations induce LTP. In between, a “no man’s land” does exist, in which no plasticity results, and very high calcium concentrations

Table 2

Results of the repeated-measures ANOVA

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Factor</th>
<th>Df</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDCS</td>
<td>Medication</td>
<td>3</td>
<td>0.596</td>
<td>0.622</td>
</tr>
<tr>
<td></td>
<td>Stimulation</td>
<td>1</td>
<td>117.900</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>14</td>
<td>1.233</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>Medication × Stimulation</td>
<td>3</td>
<td>5.050</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Medication × Time</td>
<td>42</td>
<td>0.831</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>Stimulation × Time</td>
<td>14</td>
<td>10.013</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Medication × Stimulation × Time</td>
<td>42</td>
<td>2.375</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PAS</td>
<td>Medication</td>
<td>3</td>
<td>0.838</td>
<td>0.488</td>
</tr>
<tr>
<td></td>
<td>Stimulation</td>
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<td>19.134</td>
<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>14</td>
<td>1.285</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>Medication × Stimulation</td>
<td>3</td>
<td>1.468</td>
<td>0.252</td>
</tr>
<tr>
<td></td>
<td>Medication × Time</td>
<td>42</td>
<td>0.871</td>
<td>0.699</td>
</tr>
<tr>
<td></td>
<td>Stimulation × Time</td>
<td>14</td>
<td>19.084</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Medication × Stimulation × Time</td>
<td>42</td>
<td>1.476</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

*Significant results at P < 0.05.

Effect of Varenicline on PAS-Induced Plasticity

The ANOVA revealed significant main effects of Stimulation (F1 = 19.134; P = 0.003), Stimulation × Time (F14 = 19.064; P < 0.001), and Medication × Stimulation × Time (F42 = 1.476; P = 0.035) interactions (Table 2).

Post hoc Student’s t-tests show that MEPs were significantly enhanced for about an hour after PAS25 in all medication conditions, and reduced after PAS10 in the placebo and low-dose varenicline conditions, when compared with respective baseline values. Medium and high doses of varenicline abolished PAS10-induced after-effects. Here, MEP amplitudes did not differ from baseline values at any time point, and MEPs were significantly altered when compared with the respective placebo medication conditions for up to 60 min after PAS administration. In all other conditions, MEPs obtained after varenicline administration did not differ from those under placebo medication at any time point (Fig. 3A, B).
might also prevent plasticity because of activation of hyperpolarizing potassium channels (Lisman 2001; Misonou et al. 2004). Therefore, whereas both strains of experiments stress the role of nicotine receptors for plasticity, the reason for differently directed results of animal and human experiments might be different amounts of calcium influx caused by the respective receptor agonists, and plasticity induction procedures.

The reason that low-dosage varenicline had no significant effect on plasticity is most probably that this dosage did not suffice to activate nicotinic receptors to an amount at which these induce relevant intraneuronal calcium concentration alterations. The plasticity-abolishing effects of the medium and high dosages of the drug with regard to excitability-diminishing plasticity and tDCS-induced facilitatory plasticity go in line with the results of previous studies (Grundey et al. 2012a, 2012b; Thirugnanasambandam et al. 2012), where global nicotinic receptor activation resulted in abolishment of these kinds of plasticity. Therefore, it is plausible that at least a part of the impact of nicotine on plasticity is driven by $\alpha_4\beta_2$ and $\alpha_7$ receptors. As varenicline is a full agonist of $\alpha_7$ and potent partial agonist of $\alpha_4\beta_2$ receptors, with a far greater affinity (4000- to 5000-fold) to $\alpha_4\beta_2$ when compared with $\alpha_7$ receptors (Avalos et al. 2002; Jensen et al. 2005; Mihalak et al. 2006; Rollema et al. 2010), the $\alpha_4\beta_2$ receptor might have a larger relevance for the results. Due to the above-mentioned calcium hypothesis, the most probable explanation for the abolishment of LTD-like plasticity by the medium dosage of the drug is that here nicotinic receptor activation drove calcium concentrations in the respective "no man’s lands". For the abolishment of the non-focal LTP-like plasticity induced by anodal tDCS under high-dosage varenicline, the same mechanism might apply. In contrast, the PAS25-induced excitability enhancement was not affected by any dose of varenicline. This can be explained by differences between the stimulation-inducing protocols. Neuroplastic changes via tDCS are achieved by long-lasting, tonic depolarization of large neuronal populations and activation of

![Figure 2. Impact of varenicline on tDCS-induced neuroplasticity. Shown are baseline-normalized MEP amplitudes after plasticity induction by anodal (A) and cathodal (B) tDCS under 0.1, 0.3, and 1.0 mg varenicline or placebo medication conditions up to the evening of the post-stimulation day. (A) In the placebo and 0.1-mg varenicline medication conditions, anodal tDCS induced a significant excitability elevation up to 60 min after stimulation, which was abolished by 0.3 and 1.0 mg varenicline. (B) In the placebo, 0.1- and 1.0-mg varenicline medication conditions, cortical excitability was significantly reduced after cathodal tDCS administration. This effect was abolished by 0.3 mg varenicline. Error bars indicate SEM. Filled symbols indicate significant differences of post-stimulation MEP amplitudes from respective baseline values; asterisks indicate significant differences between the drug and placebo medication conditions at the same time points (Student’s t-test, two tailed, paired samples, $P < 0.05$).](http://cercor.oxfordjournals.org/)

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voltage-dependent Ca\(^{2+}\)-channels, whereas PAS only affects small groups of neurons and causes short-lasting depolarizations. Therefore, the amount of intracellular calcium increase may be smaller with regard to PAS when compared with tDCS and not sufficient to induce significant changes in neuroplasticity (Thirugnanasambandam et al. 2012).

This mechanism does, however, not explain the re-establishment of cathodal tDCS-induced LTD-like plasticity under the high dosage of the drug. Here, it could be speculated that an antagonistic effect of varenicline on the respective nicotinic receptor, which takes place for higher dosages of the drug, resulted in reduced calcium influx, and thus a restitution of plasticity. These mechanistic explanations are, however, hypothetical at present and should be explored more directly in future studies in humans and animal models.

For the overall pattern of experimental results, varenicline applied in medium and high doses results in a focusing effect on facilitatory neuroplasticity. While preserving focal plasticity, it exerts its effect mainly by first abolishing both non-focal facilitatory and focal inhibitory plasticity, similar to global nicotinic and cholinergic system activation (Kuo et al. 2007; Thirugnanasambandam et al. 2012). Such a focusing effect might be beneficial for task performance via enhancing the signal-to-noise ratio by selectively strengthening specific task-related synapses and suppressing irrelevant, non-specific neuronal activity. This can explain the positive nicotinic effect on cognitive functions (attention, working, and episodic memory), where a stable processing of information is essential (Provost and Woodward 1991; Kumari et al. 2003; Jubelt et al. 2008; Froeliger et al. 2009; Heishman et al. 2010; Mocking et al. 2012). At the highest dose of 1-mg cathodal inhibition is restored again, which may further contribute to the signal-to-noise ratio. When using a noisy random dot paradigm, subjects under cathodal stimulation were superior in detecting coherent motion (Antal et al. 2004). LTD has also been identified as an important mechanism involved in specific forms of learning and memory.

Figure 3. Impact of varenicline on PAS-induced neuroplasticity. Shown are baseline-normalized MEP amplitudes after plasticity induction by PAS25 (A) and PAS10 (B) under 0.1, 0.3, and 1.0 mg varenicline or placebo medication conditions up to the evening of the post-stimulation day. (A) Cortical excitability was significantly elevated up to 30 min in all medication conditions after PAS25 administration. (B) In the placebo and 0.1-mg varenicline medication conditions, cortical excitability was significantly reduced up to 60 min after PAS10. 0.3 and 1.0 mg varenicline abolished the above-mentioned excitability diminution. Error bars indicate SEM. Filled symbols indicate significant differences of post-stimulation MEP amplitudes from respective baseline values; asterisks indicate significant differences between the drug and placebo medication conditions at the same time points (Student’s t-test, two tailed, paired samples, \(P < 0.05\)).
(Bear 1999; Kemp and Manahan-Vaughan 2007, 2008; Griffiths et al. 2008; Collingridge et al. 2010). It might be speculated that abolishment of certain forms LTD-like plasticity by high and medium doses of varenicline can lead to impairments of LTD-dependent forms of cognitive processes, but this has been rarely reported (Levin et al. 2006). In these cases, re-establishment of non-focal LTD-like plasticity under high-dose varenicline could also contribute to a positive nicotinic effect on LTD-dependent forms of learning and memory formation. Further behavioral experiments should be designed to explore this hypothesis.

**General Remarks**

This study demonstrates that varenicline has a prominent impact on neuroplasticity in non-smoking humans, which is similar to that of nicotine application. As mentioned above, varenicline has also been explored for therapeutic application in patients with neuropsychiatric diseases (Kem 2000; Jensen et al. 2005; Mihalak et al. 2006; Hong et al. 2011; Liu et al. 2011; Shim et al. 2012; Zesiewicz et al. 2012; Anthenelli et al. 2013). It might be speculated that the impact of the drug on neuroplasticity is involved in the respective clinical effects. From this perspective, it will be important to directly correlate the physiological results of our study with the effect of nicotinic receptor activation (\(\alpha_4\beta_2\) and possibly \(\alpha_7\) too) on cognition and clinical symptoms in future studies.

As several recently published studies demonstrate age-related changes of plasticity, the results of the study conducted in a group of healthy young subjects might be substantially different from those of older adults. The effect size of PAS-induced plasticity linearly declines across the lifespan (Muller-Dahlhaus et al. 2008; Tecchio et al. 2008). Similar results on LTD-like plasticity have been demonstrated using continuous theta burst stimulation and repetitive TMS (Todt et al. 2010; Freitas et al. 2011). Motor learning and motor performance have also been shown to decline with age (Rogasch et al. 2009; Cirillo et al. 2010, 2011). Moreover, decline in the density of nicotinic receptors with aging have been demonstrated (Schröder et al. 1991). Therefore, the impact of varenicline on cortical plasticity specifically in older adults should be studied in future experiments.

It has to be taken into account that the results of this study show the impact of only a single-dose varenicline on neuroplasticity. The effect of varenicline after chronic administration on neuroplasticity might be qualitatively different from that after an acute dose. Most of the studies with acute and/or chronic administration of nicotine in animal or human trials report a positive impact on cognitive functions (Rezvani and Levin 2001; Levin et al. 2006). However, chronic exposure to nicotine can cause upregulation (Wonnacott 1990; Buisson and Bertrand 2001; Mignaini et al. 2002; Parker et al. 2004), and desensitization of nAChRs (Hsu et al. 1996; Fenster et al. 1997, 1999), increase in brain-derived neurotrophic factor (BDNF) levels (Kenny et al. 2000), as well as affect dopaminergic (Pietila and Ahtee 2000; Rahman et al. 2004; Tan et al. 2009) and serotoninergic (Awtry and Werling 2003; Semba and Wakuta 2008) systems. Additionally, single- and double-pulse TMS protocols, such as cortical silent period, short-latency afferent inhibition (SAI) and intracortical facilitation (ICF) are affected in smokers and shifted toward cortical excitability diminution, compared with non-smoking individuals, especially under nicotine withdrawal (Lang et al. 2008), which suggests an involvement of muscarinic acetylcholine and possibly GABA\(_A\), and NMDA receptors (Inghilleri et al. 1996; Ziemann et al. 1996a, 1996b; Siebner et al. 1998; Di Lazzaro et al. 2000). This leads to an assumption that chronic administration of nicotine can result in complex changes of plasticity. Indeed, in a group of nicotine-dependent human subjects, nicotine withdrawal resulted in a complete abolishment of tDCS- and PAS-induced facilitatory plasticity, which was restored under nicotine (Gründy et al. 2012a, 2012b), whereas in a non-smoking control group, nicotine had the above-mentioned focusing effect on plasticity (Thirugnanasambandam et al. 2012).

**Figure 4.** Both anodal and cathodal tDCS-induced plasticity is abolished by 0.3 mg varenicline. Anodal tDCS-induced excitatory plasticity is abolished and cathodal tDCS-induced inhibitory plasticity is preserved by 1.0 mg varenicline. 0.1 mg varenicline has no impact on stimulation-induced plasticity. Medium and high doses of varenicline abolished PAS10-induced inhibitory plasticity. Varenicline at any doses did not have an impact on PAS25-induced excitability enhancement. Each column represents the mean of baseline-normalized MEP ± SEM amplitudes until 30 min after stimulation; asterisks indicate significant differences between drug and placebo conditions (Student’s t-test, two-tailed, paired samples, \(P < 0.05\)).
The respective mechanisms responsible for these effects should be addressed in future studies.

Since a recent study has shown that verbal and working memory functions are reduced in smoking individuals after nicotine withdrawal and restituted by nicotine re-administration (Cole et al. 2010; Grundey et al. 2012a, 2012b), and varenicline has similar effects on working memory performance in nicotine abstinence (Patterson et al. 2009; Loughead et al. 2010). It might be interesting to explore if varenicline has similar restituting effects on plasticity, as shown for cognitive processes, in these individuals.

**Limiting Conditions**

A possible limitation to our study is that varenicline is an agonist with moderate affinity to 5-HT3 serotonin receptors (Lumnis et al. 2011). 5-HT3 receptors have a facilitatory impact on plasticity (Normann et al. 2007; Nitsche et al. 2009; Batsikadze et al. 2015). However, concentrations of therapeutic unbound varenicline in the brain are insufficient for activation of these receptors (Rollemann et al. 2011). Moreover, in a recently conducted study, the serotonin reuptake inhibitor citalopram enhanced tDCS-induced LTP-like plasticity, and converted LTD-like plasticity into facilitation (Nitsche et al. 2009). These results are qualitatively different to those obtained in the present study. Varenicline has also an impact on D2/3 dopamine receptor binding and availability in rats (Crunelle et al. 2009, 2011, 2012) and GABAergic synaptic transmission (DuBois et al. 2013), which have a major impact on stimulation-induced plasticity. It should be noted that, also for these transmitters and receptors, pharmacological modulations resulted in effects which clearly differ from those obtained under varenicline (Nitsche et al. 2004; Kuo et al. 2008; Monte-Silva et al. 2009, 2010). Nevertheless, in order to explore the complex interplay of neuromodulatory systems on nicotine-modulated plasticity, future studies should use approaches combining pharmacological interventions with neuroimaging.

It should also be mentioned that the participants were not tested for BDNF and serotonin transporter gene (5-HTTLPR) polymorphisms. Global nicotine and serotonin enhancement tested for BDNF and serotonin transporter gene (5-HTTLPR) polymorphisms. Global nicotine and serotonin enhancement (Umene-Nakano et al. 2010). Since non-invasive brain stimulation-induced plasticity is affected by BDNF (Cheeran et al. 2008; Antal et al. 2010; Fritsch et al. 2010), the complex interaction between the above-mentioned factors should be explored in future studies.

Another limitation is that the specific neurophysiological mechanisms underlying the nicotinic impact on various cortico-spinal and intracortical excitability parameters were not investigated. We did not perform these measures in the present study, because this would have made it impossible to explore the detailed time course of plasticity. However, it would be important to explore the effect of varenicline on cholinergic activity, for example, by monitoring SA1 and on GABAergic and glutamatergic transmission by measuring short-latency intracortical inhibition and ICF (Ziemann et al. 1996a, 1996b; Di Lazzaro et al. 2002, 2005; Paulus et al. 2008), to unravel the physiological background of the respective effects.

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