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Cortical Hypoexcitability in Chronic Smokers? A Transcranial Magnetic Stimulation Study

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Studies in animal models and humans indicate that chronic nicotine intake influences neuronal excitability, resulting in functional and structural CNS changes. The aim of the present study was to explore human primary motor cortex (M1) excitability with transcranial magnetic stimulation (TMS) in chronic smokers. A total of 44 right-handed volunteers, aged 20–30 years, participated in the study. Chronic smokers were compared with age- and sex-matched healthy nonsmokers. We tested cortical excitability with single- and paired-pulse TMS to the left M1 and short-latency afferent inhibition (SAI) by combining median nerve stimulation and motor cortex TMS. Compared with nonsmoking controls, chronic smokers showed a significantly larger amount of SAI, which is thought to depend upon the activity of cholinergic inhibitory circuits produced by somatosensory inputs. Moreover, TMS-evoked inhibitory cortical silent periods were prolonged, whereas paired-pulse intracortical facilitation and motor-evoked potentials during moderate contraction were reduced. The results suggest that chronic nicotine intake may not only strengthen cholinergic inhibitory circuits, but could also be associated with enhanced inhibitory and reduced facilitatory mechanism of specific neuronal circuits in motor cortex. These changes may form a physiological basis for neurobiological and behavioral variations associated with chronic smoking.
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INTRODUCTION

Nicotine is the main neuroactive component of tobacco, and thus might explain some of the addictive features of tobacco consumption. However, knowledge about the specific effect of nicotine on the central nervous system is far from being complete. The functional impact of nicotine has been studied in humans and animals to some extent. On a cognitive level, acute and chronic exposure to nicotine has been shown to improve attention and working memory (Hahn and Stolerman, 2002; Kumari *et al*, 2003; Thiel *et al*, 2005). On the level of neuropsychiatric diseases, such as schizophrenia, nicotine might be used by smokers to compensate for subtle cognitive deficits. It has been shown that smokers display reduced working and verbal memory capacity compared to nonsmokers, especially under nicotine withdrawal (Jacobsen *et al*, 2005). Similarly, schizophrenic patients exhibit visuospatial working memory deficits under nicotine abstinence, which is improved relevantly by nicotine consumption (Sacco *et al*, 2005).

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Knowledge about the neurophysiological foundation of these effects of nicotine, however, is largely restricted to animal experimentation. It is known from *in vitro* animal experiments that the nicotinic acetylcholine receptor (nAChR) induces neuronal depolarization by a transmembrane cationic inward current. Hereby, calcium seems to play a dominant role (Burnashev, 1998). Since most of the cholinergic neurons do not terminate on postsynaptic sites, acetylcholine and nicotine have been characterized more as neuromodulators than transmitters (Mansvelder *et al*, 2006). nAChR modulate glutamate, dopamine, and GABA release primarily presynaptically. Nicotine also activates presynaptic non-a7 nAChRs on feed-forward interneurons and hereby decreases the evoked release of GABA onto pyramidal cells (Yamazaki *et al*, 2005). Furthermore, nicotine increases the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in pyramidal cells, and concomitantly causes a reduction in the size of responses to focal GABA application onto pyramidal cells, suggesting that the nicotine-induced increase in interneuronal activity leads ultimately to a use-dependent depression of evoked IPSCs in pyramidal cells (Yamazaki *et al*, 2005). Consequently activation of nicotine receptors can result in complex interactions involving inhibition or disinhibition of pyramidal cortical neurons (Alkondon *et al*, 2000), and the translation into functional effects is not easily done. The situation is complicated further by the fact that chronic

exposition to nicotine induces long-lasting modifications of cortical architecture, like dendritic arborization and nicotinic receptor density (Breese *et al*, 1997; Gonzalez *et al*, 2005).

The neurophysiological action of nicotine in humans is largely unknown so far. In accordance with animal experiments, enhancement of acetylcholine receptor activity by a single oral dose of the acetylcholinesterase inhibitor tacrine has been shown to decrease intracortical inhibition and increase facilitation in healthy subjects (Korchounov *et al*, 2005). However, in Alzheimer's patients, who show reduced intracortical inhibition compared to age-matched controls, treatment with the cholinesterase inhibitor donepezil increased intracortical inhibition (Liepert *et al*, 2001a). The relative contribution of nicotinic receptors to these effects was explored in only one study, where it was demonstrated that reduced intracortical inhibition and short interval afferent inhibition was normalized in patients with Tourette's syndrome after a single dose of nicotine (Orth *et al*, 2005).

To shed some more light on the impact of nicotine on cortical function in humans, we explored the influence of chronic nicotine consumption on motor cortical network excitability by comparing age- and sex-matched groups of otherwise healthy smokers and nonsmokers with transcranial magnetic stimulation (TMS). TMS gives the possibility of exploring noninvasively the mode of action of neuroactive substances in the intact human brain, and allows qualitative and quantitative evaluation of distinct neuronal networks, which might be involved in the action of nicotine. Our hypothesis was that chronic smokers would show abnormal excitability of neuronal circuits involving motor cortex compared to controls, predominant in those circuits with considerable influence of cholinergic receptor activity.

MATERIALS AND METHODS

Subjects

Altogether 44 university students (age range 20–30 years) participated in the study. General exclusion criteria were cardiac pacemaker; metal implants in the head; age younger than 18 or older than 45 years; current intake of any medication; current or previous neurological, psychiatric, or internal diseases; pregnancy or breastfeeding; current or previous drug (other than nicotine) or alcohol abuse; or participation in another clinical trial within the last 8 weeks. Chronic smokers had to be continuous and uninterrupted consumers of at least 10 cigarettes per day within the past 4 years, with a score in the Fagerstrom Test for Nicotine Dependence of 1–5 indicating mild levels of nicotine dependence (Heatherton *et al*, 1991). In order to minimize acute nicotine effects immediately after consumption as well as withdrawal effects, smokers were always ask to smoke their last cigarette 1 h before the beginning of the individual experimental session. Nonsmokers had to be without a history of continuous smoking and without occasional nicotine consumption within the past 4 years. All subjects were right-handed, according to the Edinburgh Handedness Inventory (Oldfield, 1971). The study was conducted according to the Declaration of Helsinki and approval was obtained from the Ethics Committee of the Georg-August University, Göttingen. All subjects gave their informed written consent before participation.

Data Acquisition

During the experiments subjects were comfortably seated in a reclining chair with head and arm rests. Surface electromyogram (EMG) was recorded from a right hand muscle through a pair of Ag–AgCl surface electrodes in a belly–tendon montage. Raw signals were amplified, band-pass filtered (3 Hz–3 kHz), digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK) controlled by Signal Software (Cambridge Electronic Design, version 2.13), and stored on a personal computer for offline analysis. Complete relaxation was controlled through auditory and visual feedback of EMG activity. TMS was performed by using a Magstim standard double ('figure-of-eight') 70-mm coil connected to a monophasic Magstim 200 stimulator (experiment 1) or to two Magstim 200 stimulators via a bistim module (experiment 2) (all TMS devices manufactured by the Magstim Company, Dyfed, UK). The coil was held tangentially to the skull over the left primary motor cortex (M1) with the handle pointing posterolaterally at a 45° angle to the sagittal plane. This orientation of the induced electrical field is thought to be optimal for a predominantly transsynaptic mode of activation of the corticospinal system (Di Lazzaro *et al*, 1998). At the beginning of each session, the optimal position of the TMS coil over the left M1 for eliciting MEP in the resting hand muscle was assessed. The site was marked with a skin marker to ensure that the coil was held in the correct position throughout the experiment.

Experiment 1 (Afferent Inhibition)

A total of 24 subjects participated in the first experiment. This experiment was performed in order to examine SAI and long-latency afferent inhibition (LAI), which can be determined by a conditioning-test paradigm of combined peripheral and cortical stimulation (Di Lazzaro *et al*, 2000; Tokimura *et al*, 2000). Participants for this experiment were divided into two age- and sex-matched groups of 12 smokers (10 men, 2 women; mean age 25 ± 0.57 years) and 12 nonsmoking controls (10 men, 2 women; mean age 24 ± 0.7 years). Conditioning stimuli were single electrical pulses generated by a Digitimer D185 stimulator (Digitimer Ltd., Welwyn Garden City, UK) applied through bipolar electrodes to the right median nerve at the wrist. Compared to previous studies (Di Lazzaro *et al*, 2000; Tokimura *et al*, 2000), a relatively low intensity of the conditioning stimulus (80% of the individual electric motor threshold, EMT, of the nerve) was chosen in order to avoid an influence from muscle contractions by the conditioning stimulus. The intensity of the cortical (test) stimulus was adjusted to elicit MEP in the relaxed APB with peak-to-peak amplitudes of approximately 1 mV. The conditioning stimulus to the peripheral nerve preceded the magnetic test stimulus by interstimulus intervals (ISIs) of 20, 40, 100, and 200 ms. An ISI of 20 ms can be used to study SAI, whereas 100 and 200 ms will lead to LAI. In random order the control condition (test pulse alone) was tested 30 times and each of

the conditioning-test pairs 15 times. The amplitude of the conditioned MEP was expressed as a percentage of the amplitude of the test MEP.

Experiment 2 (Motor Cortex Excitability)

A total of 38 subjects (18 of whom also took part in the first experiment) participated in the second experiment, which was performed in order to examine a variety of other TMS measurements describing inhibitory and facilitatory circuits in M1. Participants were again divided into two age- and sex-matched groups, now consisting of 19 smokers (13 men, 6 women; mean age 24 ± 0.44 years) and 19 nonsmoking controls (13 men, 6 women; mean age 24 ± 0.35 years).

TMS measurements included resting motor threshold (RMT) and active motor threshold (AMT), the TMS-intensity to evoke MEP of approximately 1-mV peak-to-peak amplitude (SI1mV), I/O curves, SICI/ICF, LICI, active MEP (aMEP) during moderate tonic activation, and the cortical silent period (CSP). Stimulus intensities (in percentage of maximal stimulator output) were determined at the beginning of each experiment, starting with SI1mV. RMT was defined as the minimal output of the stimulator that induced a reliable MEP (approximately 50 μV in amplitude) in at least 5 of 10 consecutive trials in the relaxed FDI, and AMT was defined as the lowest stimulus intensity at which 5 of 10 consecutive stimuli elicited reliable MEP (approximately 200 μV in amplitude) in the tonically contracted FDI (Rothwell *et al*, 1999). I/O curves were measured with three different stimulus intensities (100, 120, and 140% RMT), each with 10 pulses. A mean was calculated for each intensity. SICI/ICF and LICI were measured with different protocols of single- and paired-pulse TMS applied at 0.25 Hz. For SICI/ICF, two magnetic stimuli were given through the same stimulating coil, and the effect of the first (conditioning) stimulus on the second (test) stimulus was investigated (Kujirai *et al*, 1993). To avoid floor or ceiling effects, the intensity of the conditioning stimulus was set to 90% AMT. The test-stimulus intensity was adjusted to SI1mV. SICI/ICF was measured with ISIs of 2, 4, 7, 9, and 12 ms. The control condition (test pulse alone) was tested 40 times, each of the conditioning-test pairs 20 times, and conditions were applied in randomized order. The mean amplitude of the conditioned MEP at each ISI was expressed as a percentage of the mean size of the unconditioned test pulse. SICI was taken as the mean percentage inhibition at ISIs of 2 and 4 ms, whereas ICF was taken as the mean facilitation at ISIs of 9 and 12 ms. Another protocol tested LICI with two suprathreshold stimuli applied with ISIs of 50, 100, and 150 ms (Valls-Sole *et al*, 1992). The intensity of both stimuli was set to the relatively low value of 110% RMT to avoid floor effects. The control condition (first pulse alone) was tested 30 times, each of the paired stimuli 15 times, and conditions were applied in randomized order. LICI was taken as the mean percentage inhibition of conditioned MEP at ISIs of 50, 100, and 150 ms. At the end of each session, 10 pulses with SI1mV and 120% RMT, respectively, were applied during moderate tonic contraction of the FDI (approximately 30–50% of maximal voluntary contraction). The mean peak-to-peak MEP amplitude (in mV) was taken from each intensity for aMEP. CSPs were separately determined in rectified and averaged EMG traces with a prestimulus period of 100 ms. CSP (in ms) was measured from the TMS stimulus artifact to the point where the signal reached the amplitude of the mean prestimulus EMG activity again for >5 ms.

Data Analyses

For both experiments and each measure (SAI/LAI, SI1mV, RMT, AMT, I/O curve, SICI/ICF, LICI, aMEP, and CSP), we performed separate analyses of variance (ANOVAs) for repeated measurements by using mean values from each subject as the dependent variable. In addition to the factor 'group' (smokers *vs* nonsmokers), the ANOVA model included the factor 'ISI' (20, 40, 100, and 200 ms) when SAI/LAI was analyzed, or 'ISI' (2, 4, 7, 9, and 12 ms) when SICI/ICF was analyzed, or the factor 'intensity' (100, 120, and 140% RMT) for I/O curves, or 'intensity' (120% RMT and SI1mV) for CSP and aMEP. Paired-samples two-tailed *t*-tests were used to compare thresholds (EMT, RMT, AMT) and TMS intensities (SI1mV) between groups in both experiments. The Greenhouse–Geisser method was used when necessary to correct for nonsphericity. Conditional on a significant F-value in ANOVA, paired-samples two-tailed *t*-tests were used for *post hoc* analysis, and a *p*-value of <0.05 was considered significant for all statistical analyses. Data are expressed as mean ± SEM.

RESULTS

Fagerstrom Scores

Mean Fagerstrom score of the smoking groups were 2.92 ± 0.4 in the first experiment (afferent inhibition) and 3.19 ± 0.24 in the second experiment (motor cortex excitability). This indicates low levels of nicotine dependence in both experiments.

Stimulation Parameters

Mean stimulation parameters from both experiments are given in Table 1. Paired-samples *t*-tests did not detect significant differences for peripheral nerve and cortex stimulation parameters between groups (experiment 1: EMT $t = 0.369$, $df = 11$, $p = 0.536$; and SI1mV $t = 0.421$, $df = 11$,

Table 1 Mean Values of Electric Motor Threshold, Stimulus Intensity Necessary to Evoke MEP of 1-mV Amplitude, Resting Motor Threshold, and Active Motor Threshold from Both Experiments

	Experiment 1		Experiment 2		
	EMT[a]	SI1mV[b]	RMT[b]	AMT[b]	SI1mV[b]
Smokers	144 ± 19	45 ± 3	51 ± 2	37 ± 1	63 ± 2
Nonsmokers	153 ± 13	48 ± 3	50 ± 1	37 ± 1	61 ± 2

Abbreviations: AMT, active motor threshold; EMT, electric motor threshold; RMT, resting motor threshold; SI1mV, 1-mV peak-to-peak amplitude.
Paired-samples *t*-test did not reveal significant differences between groups (smokers *vs* nonsmokers) for any of these parameters. ([a]in volts; [b]in % of maximal stimulator output).

$p = 0.682$; experiment 2: RMT $t = 0.316$, df = 18, $p = 0.756$; AMT $t = 0.306$, df = 18, $p = 0.764$; and SI1mV $t = 0.558$, df = 18, $p = 0.583$).

Afferent Inhibition

Smokers showed significantly more SAI compared to nonsmoking controls, whereas LAI did not differ between groups (Figure 1). ANOVA on mean SAI/LAI values demonstrated a significant main effect for the factor 'ISI' ($F(3, 33) = 14.6$, $p < 0.001$) and a significant interaction 'group' by 'ISI' ($F(3, 33) = 3.2$, $p = 0.038$). *Post hoc* analysis revealed a significant difference between groups at ISI 20 ms, indicating that SAI at this interval was more pronounced in the smokers group compared to nonsmokers (*post hoc t*-test: $t = 4.093$, df = 11, $p = 0.002$).

Motor Cortex Excitability

At rest, smokers demonstrated reduced ICF compared to nonsmokers (Figure 2). ANOVA on SICI/ICF showed a trend for the factor 'group' ($F(1, 18) = 3.6$, $p = 0.074$), a significant effect for the factor 'ISI' ($F(3, 60) = 52.1$, $p < 0.001$), and a significant interaction 'group' by 'ISI' ($F(3, 55) = 3.3$, $p = 0.025$). *Post hoc* analyses demonstrated that smokers had significantly less ICF at ISI 9 ms ($t = -2.289$, df = 18, $t = 0.034$) and 12 ms ($t = -3.272$, df = 18, $p = 0.004$) compared with nonsmokers. Thus, mean ICF, expressed as an arithmetic mean of ICF at 9 and 12 ms, was significantly reduced in smokers ($t = -2.924$, df = 18, $p = 0.009$). Figure 3 illustrates data from parameters obtained during voluntary activation of the motor system (CSP and aMEP). ANOVA on CSP showed a significant main effect for the factor 'group' ($F(1, 18) = 4.8$, $p = 0.042$) and for 'intensity' ($F(1, 18) = 7.7$, $p = 0.013$), but not for an interaction of the two factors. This indicates differences in CSP between groups as well as between intensities, and that differences in CSP between groups were independent of intensities used to evoke CSP. Differences between intensities can be explained by a well-known positive correlation of CSP duration with stimulus intensity (Haug *et al*, 1992). *Post hoc t*-tests demonstrated a significant prolongation of CSP in smokers compared to nonsmokers when SI1mV was used as intensity ($t = 2.402$, df = 18, $p = 0.027$) and a trend with 120% RMT ($t = 1.858$, df = 18, $p = 0.082$). ANOVA on aMEP revealed a significant main effect for the factor 'group' ($F(1, 18) = 5.9$, $p = 0.026$), but not for the factor 'intensity' and no interaction of the two factors. This indicates that differences in aMEP between groups were independent of intensities used to evoke aMEP. *Post hoc t*-tests demonstrated a significant reduction of aMEP in smokers compared to nonsmokers when SI1mV was used as intensity ($t = -2.052$, df = 18, $p = 0.015$) and a trend with 120% RMT ($t = -2.687$, df = 18, $p = 0.055$). Apart from an inherent effect for 'intensity' on I/O curve values ($F(2, 36) = 67.6$, $p < 0.001$) separate ANOVAs on LICI and

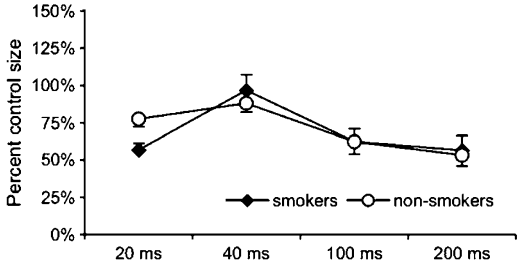

Figure 1 Increased short-latency afferent inhibition in smokers. Afferent inhibition was tested at rest by combining median nerve stimulation and motor cortex TMS with interstimulus intervals of 20 ms (short-latency afferent inhibition), 40 ms (indifferent interval), as well as 100 and 200 ms (long-latency afferent inhibition). Amplitudes of the conditioned MEP are expressed as a percentage of the amplitudes of the test MEP (TMS alone). Data are represented as mean ± SEM. SAI at 20 ms was found significantly more pronounced in smokers compared to nonsmokers (ANOVA, *post hoc t*-test; for details see Results), whereas LAI did not differ between groups.

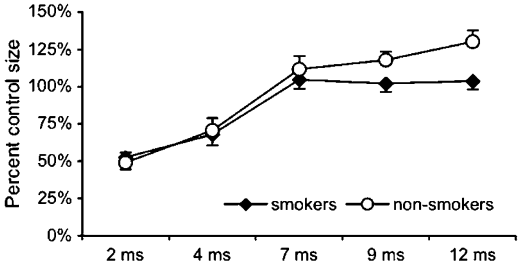

Figure 2 Reduction of intracortical facilitation in smokers. Short-latency intracortical inhibition and facilitation was tested at rest with paired-pulse motor cortex TMS at interstimulus intervals of 2–12 ms. Amplitudes of the conditioned MEP are expressed as a percentage of the amplitudes of the test MEP (single TMS pulse). Data are represented as mean ± SEM. Intracortical facilitation at interstimulus intervals 9 and 12 ms was found significantly reduced in smokers compared to nonsmokers (ANOVA, *post hoc t*-test; for details see Results), whereas short-latency intracortical inhibition did not differ between groups.

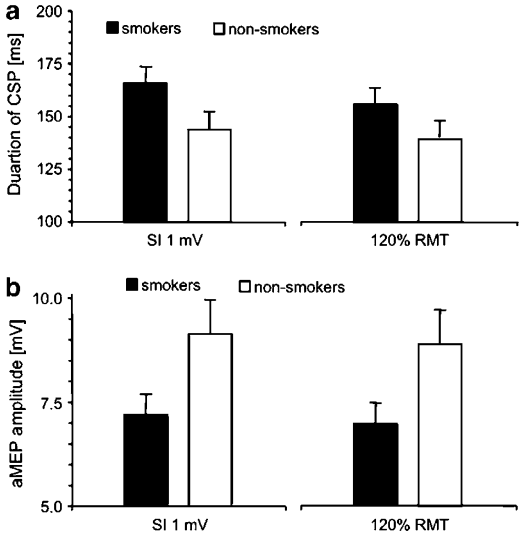

Figure 3 Increased inhibition and reduced facilitation during motor system activation in smokers. Cortical silent periods (CSP, a) and active MEP (aMEP, b) were evoked with two different stimulus intensities (1-mV peak-to-peak amplitude (SI1mV) and 120% resting motor threshold (RMT)) during moderate contraction of the target muscle. Data are represented as mean ± SEM. The duration of CSP was found significantly prolonged and aMEP amplitude reduced in smokers compared to nonsmokers, irrespective of the mode of measure (ANOVA, *post hoc t*-test, for details see Results).

I/O curve values revealed no significant main effects or interactions, and motor thresholds or stimulation intensities did not differ between groups (Table 1).

DISCUSSION

The present study investigated human motor cortex excitability in chronic smokers with TMS, an approach with advantages and limitations. Advantages include the possibility of exploring noninvasively inhibitory and excitatory cortical networks in the intact human under chronic nicotine exposure and comparing the pattern of excitability with those induced by pharmacological agents or found in neuropsychiatric disorders. In contrast to most animal research, TMS can describe alterations at the system level of the human cerebral cortex, often being more closely related to the clinical context. Potential limitations of the present study may be that the observed effects could partially be a reflection of acute nicotine administration rather than the effect of chronic smoking, as well as that the study design did not control completely for a trait that predisposes to nicotine consumption. We attempted to reduce the problem of acute nicotine administration to a minimum by performing the experiments within the second hour after smoking cessation, hence to the latest possible time point before relevant withdrawal symptoms would arise. Personality traits can influence TMS measures of motor cortical excitability, as has been shown ie in anxiety (Wassermann *et al*, 2001). However, the fact that chronic smokers in our study showed only low nicotine dependence reduces this weakness.

Despite this limitation, the present study supplies novel insights into cerebral cortex physiology of chronic smokers. It demonstrates that chronic smokers differ from nonsmoking individuals with regard to motor-cortical excitability tested with TMS. Specifically, smokers display enhanced short-latency afferent inhibition (SAI), prolonged inhibitory silent periods, reduced intracortical facilitation and lower aMEP amplitudes. This pattern of results argues for specific differences of excitability between both groups on the *cortical* level. While the above-mentioned protocols test different intracortical neuronal circuits, corticospinal excitability, as tested by the I/O curve, the TMS intensity necessary to elicit 1-mV MEP amplitudes, and motor thresholds were identical between the groups. Also, no difference could be found between groups regarding LAI, which probably involves the basal ganglia–thalamocortical loop (Sailer *et al*, 2003).

The aMEP amplitude is a global measure of motor-cortical excitability, which is influenced by ion channel-activity, GABAergic, glutamatergic, dopaminergic, adrenergic, and cholinergic receptors (for review see Ziemann, 2003). The reduction of MEP amplitudes in smokers is in clear favor for enhanced motor-cortical inhibition, but the multitude of mechanisms involved, added by the indirect modulatory effects of nicotine on diverse types of receptors does not allow to derive definite conclusions about the specific mechanism how nicotine reduces motor cortex excitability from these results.

However, SAI is known to reflect specifically the inhibitory effect of somatosensory afferents to the M1 (Tokimura *et al*, 2000). In a patient with paramedian thalamic stroke absence of SAI could be demonstrated on the affected side, suggesting that thalamocortical projections from the paramedian thalamus contribute to the integration of sensory input at the cortical level (Oliviero *et al*, 2005). The inhibitory action of SAI seems to be cholinergically driven and it was demonstrated that it is reduced by the muscarinic receptor antagonist scopolamine (Di Lazzaro *et al*, 2000). The results of our study are in favor for an additional influence of nicotine on this neuronal circuit, ie that in smokers nicotine might enhance the somatosensory inhibition of the M1 by activation of cholinergic synapses, which can be due to activation of nicotinic receptors or by the indirect activation of the muscarinic receptors. Another motor-cortical inhibitory mechanism, which is measured by the CSP, reflects a long-lasting intracortical inhibition mediated by $GABA_B$ receptors (Inghilleri *et al*, 1996; Siebner *et al*, 1998). Our results demonstrate that, like SAI, CSP is enhanced in smokers. Since nicotine is known to enhance the expression of $GABA_B$ receptors (Li *et al*, 2004), this is one likely explanation for the prolongation of CSP our smokers group. Interestingly also intracortical facilitation is, among others, modulated by $GABA_B$-receptor activity (Ziemann *et al*, 1996). Its reduction in the smokers group might thus also be due to an enhanced expression of these receptors induced by nicotine.

A possible alternative explanation for the present observations is that nicotine activates cholinergic circuits via muscarinic receptors. This would explain the SAI increase and MEP amplitude reduction, which is the opposite finding of the work by Di Lazzaro *et al* (2000) with blocking muscarinic receptors. Nicotinic, but not muscarinic receptors could also activate an alternative, non-GABAergic pathway that could explain the CSP prolongation.

However, although these mechanisms of function seem plausible, it should be kept in mind that the TMS protocols tested here are not restricted to the influence of one type of receptor. For example, dopaminergic drugs enhance CSP duration and reduce ICF, and NMDA receptors are also involved in ICF generation (for review see Ziemann, 2004). Given the neuromodulatory action of nicotine on diverse receptor types, alternative mechanisms of action are conceivable. This is underlined by the fact that the $GABA_B$-agonist baclofen has been described to enhance SICI elsewhere (Ziemann *et al*, 1996), but SICI was identical for both subject groups in our study. Thus, the hypothesis of an effect of nicotine restricted to cholinergic receptors and $GABA_B$ activity seems questionable. Future studies should explore this to a larger extent.

Our results are not in full accordance with those of former studies. Enhancement of acetylcholine by a single oral dose of tacrine reduced SICI and enhanced ICF in healthy subjects in another study (Korchounov *et al*, 2005), and a similar effect was achieved by a single dose of the muscarinic antagonist atropine (Liepert *et al*, 2001b). Moreover, application of a cholinesterase antagonist in Alzheimer's patients and of nicotine in patients with Tourette's syndrome increased SICI (Orth *et al*, 2005). This seemingly opposing effects of ACh modulation in different studies underline the fact that it acts as a neuromodulator and thus the direction of its action might critically depend not only on the subreceptors involved, but also on receptor

availability, baseline activation, chronic *vs* acute administration, and other factors.

As mentioned above, a possible limitation of this study is that we did not look directly for causality, that is we did not modulate nicotine in our subjects and therefore it cannot be concluded clearly if the cortical excitability differences between the groups under study are caused by nicotine consumption or are representing a kind of proneness for nicotine dependency in the smokers group. The situation is even more complicated by the fact that chronic exposition to nicotine leads to an enhancement and desensitization of nicotinic, but to enhanced affinity of muscarinic receptors to acetylcholine (Breese *et al*, 1997; Flores *et al*, 1992; Schwartz and Kellar, 1985; Wang and Sun, 2005) and to structural cortical alterations (Gonzalez *et al*, 2005). Thus, the complex interplay between inherent cortical excitability differences between smoking and nonsmoking individuals, its modification by chronic nicotine consumption, and the acute effects of nicotine should be taken into account in future studies.

Taken together, the results of the current study show a cortical excitability reduction in smokers as compared to nonsmoking individuals. It might be speculated that this functional cortical modification is due to chronic nicotine consumption. A possible functional relevance of enhanced cortical inhibition might be a reduction of cortical noise, thus enhancing the salience of stimuli-associated information processing. Indeed, acetylcholine has been proposed to increase cortical signal-to-noise ratio and to improve cognitive functioning (Gu, 2003). This mechanism might also be relevant for diseases displaying pathologically reduced inhibition or signal-to-noise ratio, as in Tourette's syndrome or schizophrenia.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except income received from primary employers, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal finance holdings that could be perceived as constituting a potential conflict of interest.

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