Smoking Restores Impaired LTD-Like Plasticity in Schizophrenia: a Transcranial Direct Current Stimulation Study

Wolfgang Strube[1], Tilmann Bunse[1], Michael A Nitsche[2], Thomas Wobrock[3,4], Richard Aborowa[4], Kristina Misewitsch[4], Maximiliane Herrmann[4], Peter Falkai[1] and Alkomiet Hasan*,[1]

[1]Department of Psychiatry and Psychotherapy, Ludwig Maximilian University, Munich, Germany; [2]Department of Clinical Neurophysiology, University of Goettingen, Goettingen, Germany; [3]Centre of Mental Health, Darmstadt-Dieburg Clinics, Darmstadt, Germany; [4]Department of Psychiatry and Psychotherapy, University of Goettingen, Goettingen, Germany

Impaired neuroplastic responses following noninvasive brain stimulation have been reported repeatedly in schizophrenia patients. These findings have been associated with deficits in GABAergic, glutamatergic, and cholinergic neurotransmission. Although various neurophysiological studies have indicated a relationship between nicotine and neuroplasticity in healthy individuals, the present study is the first investigation into the impact of nicotine on LTD-like plasticity in patients with schizophrenia. Cortical excitability and cortical plasticity were explored in 30 schizophrenia patients (17 smoker, 13 nonsmoker) and 45 healthy controls (13 smoker, 32 nonsmoker) by using single-pulse transcranial magnetic stimulation (TMS) before and following cathodal transcranial direct current stimulation (tDCS) applied to the left primary motor cortex. Our analysis revealed abolished LTD-like plasticity in nonsmoking schizophrenia patients. However, these plasticity deficits were not present in smoking schizophrenia patients. In healthy controls, significant MEP reductions following cathodal tDCS were observed in nonsmoking individuals, but only trend-level reductions in smokers. In smoking schizophrenia patients, the severity of negative symptoms correlated positively with reduced neuroplasticity, whereas nonsmoking patients displayed the opposite effect. Taken together, the data of our study support the notion of an association between chronic smoking and the restitution of impaired LTD-like plasticity in schizophrenia patients. Although replication and further research are needed to better understand this relationship, our findings indicate that nicotine intake might stabilize the impaired inhibition–facilitation balance in the schizophrenic brain through a complex interaction between cortical plasticity, and GABAergic and cholinergic neurotransmission, and might explain the reduced prevalence of negative symptoms in this population.

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INTRODUCTION

Severe mental illness is highly associated with an increased prevalence of cigarette smoking and tobacco dependency. Specifically, elevated average smoking rates >60% have been reported in patients suffering from schizophrenia (Dome *et al*, 2010). Different theories implicate nicotine intake via smoking as a compensatory strategy used by schizophrenia patients to ameliorate their symptoms (D'Souza and Markou, 2012).

Indicating complex alterations of the cholinergic system, rodent schizophrenia models revealed reduced α_7-nACh-receptor (nAChR) expression levels and an increased release of acetylcholine (Hashimoto *et al*, 2008a; Nelson *et al*, 2002).

Accordingly, postmortem studies and pharmacological challenges have reported further evidence of decreased expression levels of α_7-nAChR in patients with schizophrenia (D'Souza and Markou, 2012; Martin-Ruiz *et al*, 2003). At the behavioral level, the stimulation of α_7-nAChR through nicotine intake has been demonstrated to improve working memory and selective attention deficits, and to enhance thalamocortical functional connectivity in schizophrenia patients (Jacobsen *et al*, 2004; Sacco *et al*, 2005). Taken together, an illness-related dysfunction of cortico-subcortical cholinergic networks in patients with schizophrenia, involving a complex interplay of cholinergic activation and neuroplasticity, can be assumed (D'Souza and Markou, 2012).

The role of the most prominent nAChR agonist, nicotine, for neuroplasticity is evident from animal research indicating a prominent influence on plasticity functions (Levin *et al*, 2006), eg, by showing nicotine-dependent facilitation of long-term potentiation (LTP) (Huerta and Lisman, 1995), a reversal of stress-induced long-term-depression (LTD) deficits (Aleisa *et al*, 2006), and the involvement of different

nAchR subtypes in the induction of hippocampal LTD (Nakauchi and Sumikawa, 2014). Although most investigations of the nicotinergic impact on neuroplasticity are restricted to animal studies, neurophysiological studies using transcranial direct current stimulation (tDCS) have recently given insight into the potential impact of nicotine on neuroplasticity at the system level of the human cortex. Cathodal tDCS has been demonstrated to induce long-lasting and polarity-dependent changes in motor-cortical excitability, leading to the activation of physiological mechanisms related to molecular long-term-depression (LTD) (Nitsche et al, 2008). Studies applying cathodal tDCS in healthy nonsmokers for plasticity induction showed either a reduction or even an abolishment of LTD-like plasticity following prolonged and fast-acting nicotine application (Grundey et al, 2012a; Thirugnanasambandam et al, 2011). Further investigations on chronic smokers during nicotine withdrawal revealed unaffected LTD-like plasticity following cathodal tDCS, which was abolished following nicotine reapplication (Grundey et al, 2012b). Moreover, physiological investigations with transcranial magnetic stimulation (TMS) indicate that chronic smoking tilts the balance between facilitation and inhibition towards cortical hypoexcitability (Grundey et al, 2013; Lang et al, 2008). In summary, evidence points to a significant modulation of both neuroplasticity and cortical excitability through acute and chronic nicotine intake in healthy smokers and nonsmokers.

Despite the consistent reduction of cortical plasticity in schizophrenia patients following different types of plasticity-inducing non-invasive brain stimulation (Hasan et al, 2013a; Daskalakis et al, 2008a; Frantseva et al, 2008; Oxley et al, 2004) and the association of smoking with functional and symptomatic improvements (D'Souza and Markou, 2012), the impact of nicotine intake via smoking on neuroplasticity in schizophrenia has not yet been investigated. To determine the physiological basis of the association between nicotine and neuroplasticity, we applied LTD-like plasticity-inducing cathodal tDCS to smoking and nonsmoking schizophrenia patients. Given the well-established inhibitory deficit in schizophrenia in various brain regions, including the primary motor cortex (Hashimoto et al, 2008b), the positive impact of nicotine in pronouncing inhibitory functions (Lang et al, 2008), and the concept of smoking-related self-medication, we hypothesized that smoking schizophrenia patients would display fewer deficits in LTD-like plasticity compared with nonsmoking patients. On the basis of previous publications (Grundey et al, 2012b), we further hypothesized that healthy smokers and nonsmokers would display differences in LTD-like plasticity following cathodal tDCS. We also hypothesized that smoking and nonsmoking healthy subjects would show different plasticity responses compared with the respective schizophrenia patients.

MATERIALS AND METHODS

Subjects

Thirty schizophrenia patients and 45 healthy controls (aged 18–40 years) from previous studies (Hasan et al, 2012a; Hasan et al, 2013b; Hasan et al, 2012b) were pooled and included in this study, resulting in a total sample size of 75. The sample recruitment was conducted from 2009 to 2012. Participants with contraindication to TMS or tDCS were excluded. A clinical psychiatrist and a member of the study group (TW/AH) made an ICD-10 consensus diagnosis. All participants underwent a standardized test of hand preference (Oldfield, 1971), and patients received an assessment of psychopathological symptoms (PANSS), disease severity (CGI), and functioning (GAF). The ethics committee of the UMC Goettingen approved the protocol, which was conducted in accordance with the Declaration of Helsinki.

Sociodemographic variables are presented in Table 1 and concomitant medication is presented in Supplementary Table S1.

TMS

The complete protocol has been described previously (Hasan et al, 2012b; Hasan et al, 2011). Electromyographic activity (EMG) was recorded by surface electrodes on the right dorsal interosseus muscle in a belly-tendon montage. Raw signals were amplified, bandpass-filtered (2 Hz–10 kHz) and digitized using a standard amplifier (Keypoint portable, Medtronic Co., Denmark). TMS was performed over the left primary motor cortex (M1) with a standard 70-mm-TMS figure-of-eight coil and a MagPro-X-100 stimulator (Medtronic, Denmark). Throughout all the experiments, the coil was held tangentially to the head, with the handle pointing backwards and in a 45° angle lateral to the midline. The stimulation site that produced the largest motor-evoked potential (MEP) at moderately suprathreshold stimulation intensities was marked as the optimal coil position.

tDCS

Cathodal transcranial direct current stimulation (tDCS) was applied after baseline TMS examination. The tonic electrical field with a stimulation intensity of 1 mA was induced by a CE-certified stimulator (NeuroConn, Ilmenau, Germany) through saline-soaked rectangular surface sponge electrodes (35 cm^2) for a duration of 9 min. These parameters have been consistently shown to be well suited for the induction of cortical excitability alterations lasting for about 1 h after tDCS (Nitsche et al, 2008). The motor cortex electrode was placed over the left motor cortex (M1) over the location of the optimal TMS coil position for eliciting motor-evoked potentials of the target muscle and the return electrode was placed over the contralateral supraorbital forehead.

Cortical Excitability

We measured different parameters of cortical excitability (expressed as changes in MEP size) within one experimental session at baseline and one within the first 5 min following cathodal tDCS. In the case of smokers, data were acquired after at least half an hour of nicotine withdrawal. Resting motor threshold (RMT), expressed as percentage of maximum stimulator output, was defined as the lowest intensity that produced a minimum MEP of 50 μV in the relaxed FDI in at least 5 of 10 trials. To monitor after-effects of cathodal tDCS on cortico-spinal excitability, single-pulse

Table 1 Demographic and Clinical Characteristics of the Participants

Variable	SZ nonsmoker	SZ smoker	HC nonsmoker	HC smoker	Statistics (p-value)
Gender	4 F, 9 M	5 F, 12 M	13 F, 19 M	5 F, 8 M	0.848[a]
Age	28.3 ± 7.9	29.2 ± 5.4	27.4 ± 3.6	27.9 ± 4.6	0.703[b]
Handedness	11 R, 2 L	14 R, 3 L	30 R, 2 L	11 R, 2 L	0.616[a]
Fagerstroem-score	—	5.6 ± 1.5	—	2.5 ± 1.9	<0.001*,[b]
PANSS scores					
Total	59.6 ± 8.9	57.9 ± 16.8	—	—	0.739[b]
Positive	12.8 ± 4.5	14.7 ± 5.5	—	—	0.343[b]
Negative	18.7 ± 2.9	15.4 ± 5.1	—	—	0.048*,[b]
General	28.1 ± 4.9	27.8 ± 8.9	—	—	0.982[b]
GAF	59.2 ± 12.7	55.4 ± 10.5	—	—	0.375[b]
CGI	4.3 ± 1.1	4.4 ± 0.9	—	—	0.775[b]
CPZ (daily)	430.3 ± 369.9	402.9 ± 342.5	—	—	0.836[b]
Duration of illness (years)	2.7 ± 2.5	4.8 ± 4.1	—	—	0.095[b]

Abbreviations: CGI, clinical global impression; CPZ, chlorpromazine equivalent dose; GAF, global assessment of functioning; HC, healthy controls; PANSS, positive and negative syndrome scale; SZ, schizophrenia patients.
Data are presented as mean ± SD.
[a]Chi-square test.
[b]One-way ANOVA (*$p \leqslant 0.05$, #$p \leqslant 0.09$).

MEPs were recorded from the M1 representation of the right FDI. Stimulation intensity was adjusted at baseline to evoke MEPs of an average size of 1 mV (S1mV), and was then kept unchanged for the assessments after tDCS. CSP duration was recorded as FDI muscle activity under voluntary contraction while stimulating the contralateral M1 using 120% RMT. The mean CSP duration was then calculated from 10 consecutive trials. The active MEP (aMEP) was measured in 10 trials as peak-to-peak measurement.

Statistics

For statistical analyses, SPSS 22 was used. Level of significance was set at $\alpha = 0.05$. One-way analysis of variance (ANOVA) and Chi-square tests were used to analyze differences between the study groups with regard to demographic variables (age, gender, handedness). Chi-square tests were used to compare the amount of concomitant medication. Age, Fagerstroem scores and baseline excitability parameters were also compared with one-way ANOVAs. Independent t-tests were used to compare clinical measures (GAF, CGI, PANSS, duration of illness) in the patient groups. The dependent variables were RMT, S1mV, MEP size, CSP, and active MEP (aMEP). Due to the difference in sample sizes, tests for normal distribution were conducted separately for the schizophrenia and healthy control groups. Since the normality assumption was violated for RMT, S1mV, 1 mV-MEP, and aMEP in both study groups (Shapiro–Wilk-test, schizophrenia: p-values between <0.001 and 0.038; healthy controls: p-values between <0.001 and 0.047), a logarithmic transformation (ln) was applied to the raw values included in further analysis. Repeated-measure-ANOVAs (RM-ANOVA) were computed with 'time' (before *vs* after tDCS) as within-subject factor, and 'smoking status' (smoking *vs* nonsmoking) and 'study group' (schizophrenia patients *vs* healthy controls) as between-subject factors. In the event of significant interactions, paired t-tests were conducted for within-group comparisons and independent t-tests in the case of between-group pre-post comparisons (all two-tailed, $p < 0.05$, not adjusted for multiple comparisons) to analyze differences between the subgroups. Sphericity was tested with the Mauchly's test and, if necessary, the Greenhouse–Geisser correction was used. Pearson correlations between dependent variables and clinical variables were performed.

RESULTS

Sociodemographic and Clinical Characteristics

The study groups did not differ significantly with regard to gender distribution ($p = 0.848$), handedness ($p = 0.616$), or age ($p = 0.703$). According to PANSS measures, all schizophrenia patients suffered from moderate positive and negative symptoms and showed similar degrees of illness (CGI) and impairments of global functioning (GAF). Nonsmoking schizophrenia patients presented with significantly more intensive negative symptoms ($p = 0.048$) and showed a trend-level shorter duration of illness ($p = 0.095$). Mean Fagerstroem scores differed between smoking schizophrenia patients (5.6 ± 1.5) and smoking healthy controls (2.5 ± 1.9) ($p < 0.001$), indicating a higher level of cigarette consumption and tobacco dependence in schizophrenia patients. Chlorpromazine equivalents ($p = 0.836$; Table 1) and the amount of concomitant medication ($p = 0.193$; Supplementary Table S1) did not differ between the patient groups.

Cortical Excitability at Baseline

Four group comparisons of cortical excitability parameters at baseline were conducted using a one-way ANOVA and showed no significant differences across groups but trendwise differences for RMT and S1mV (Table 2).

Table 2 ANOVA of Cortical Excitability Parameters of the Dependent Variables at Baseline

Variable	SZ nonsmoker	SZ smoker	HC nonsmoker	HC smoker	ANOVA (p-value)
RMT (%)	59.0 ± 8.0	52.2 ± 10.7	50.8 ± 9.7	49.6 ± 8.9	0.054[#]
S1mV (%)	69.1 ± 9.8	61.0 ± 12.3	60.0 ± 13.5	59.0 ± 8.0	0.070[#]
MEP (mV)	994.0 ± 332.4	932.1 ± 252.5	956.0 ± 283.7	984.0 ± 291.3	0.963
CSP (ms)	149.8 ± 46.5	140.8 ± 31.5	136.1 ± 32.7	134.4 ± 36.2	0.768
aMEP (mV)	4742.1 ± 2856.9	5512.6 ± 5058.1	5412.2 ± 4101.2	4332.4 ± 3426.3	0.955

Abbreviations: HC, healthy controls; SZ, schizophrenia patients.
Data are presented as mean ± SD ($*p \leqslant 0.05$, $\#p \leqslant 0.09$).

Table 3 RM-ANOVA across all Study Groups Showed a Significant 'Time' and 'Time × Smoking-state × Study-group' Interactions for 1 mV-MEPs ($*p \leqslant 0.05$)

RM-ANOVA	Hypothesis df, error df	F-value	p-value
1 mV-MEP			
Time	1, 71	16.792	<0.0001*
Time × smoking status	1, 71	1.730	0.193
Time × group	1, 71	1.102	0.297
Time × group × smoking status	1, 71	5.479	0.022*

Figure 1 MEP size before and after cathodal tDCS. (a) Displays MEP sizes before and after cathodal tDCS in healthy nonsmokers and smokers. Dependent t-tests showed a significant MEP decrease for healthy nonsmokers ($t_{(31)} = 5.501$, $p < 0.0001$) and a trend-level difference in healthy smokers ($t_{(12)} = 1.913$, $p = 0.079$). (b) Displays MEP sizes before and after cathodal tDCS in schizophrenia nonsmokers and smokers. A significant MEP reduction was found in the smoking schizophrenia patients ($t_{(16)} = 2.464$, $p = 0.025$), but not in the nonsmoking schizophrenia patients ($t_{(12)} = 0.294$, $p = 0.774$). Instead, the latter displayed an opposite numeric increase in 1 mV-MEP size following LTD-inducing stimulation. Data are presented as mean ± SEM and graphs show untransformed data.

1 mV-MEP before and after Cathodal tDCS

The RM-ANOVA with the factors 'time', 'smoking status', and 'study group' revealed a significant main effect of 'time' ($F = 16.79$, $p < 0.0001$) and a significant 'time × smoking status × study-group' interaction ($F = 5.48$, $p = 0.022$) (Table 3). Paired t-tests (within-subject comparisons) to compare MEP sizes before and after cathodal tDCS showed a significant decrease in MEP size in healthy nonsmokers ($t_{(31)} = 5.501$, $p < 0.0001$) and a trend-level decrease in healthy smokers ($t_{(12)} = 1.913$, $p = 0.079$). Nonsmoking schizophrenia patients displayed no decrease in MEP size ($t_{(12)} = 0.294$, $p = 0.774$), whereas smoking schizophrenia patients showed a significant decrease in MEP size following cathodal tDCS ($t_{(16)} = 2.464$, $p = 0.025$; Figure 1).

Independent-samples t-tests (between-subject comparisons) comparing baseline MEP size revealed neither significant differences between healthy nonsmokers and smokers ($t_{(43)} = 0.342$, $p = 0.773$), nor between schizophrenic nonsmokers and smokers ($t_{(28)} = 0.385$, $p = 0.702$). Independent-samples t-tests also revealed no baseline MEP amplitude differences between healthy and schizophrenic nonsmokers ($t_{(43)} = 0.257$, $p = 0.798$), as well as no significant differences between healthy and schizophrenic smokers ($t_{(28)} = 0.496$, $p = 0.624$). After cathodal tDCS, independent-samples t-tests showed no significant differences between healthy smokers and nonsmokers ($t_{(43)} = 1.194$, $p = 0.239$), but did reveal significant differences between schizophrenic smokers and nonsmokers ($t_{(28)} = 2.074$, $p = 0.047$). Comparisons between healthy and schizophrenic nonsmokers revealed significant differences ($t_{(43)} = 2.936$, $p = 0.0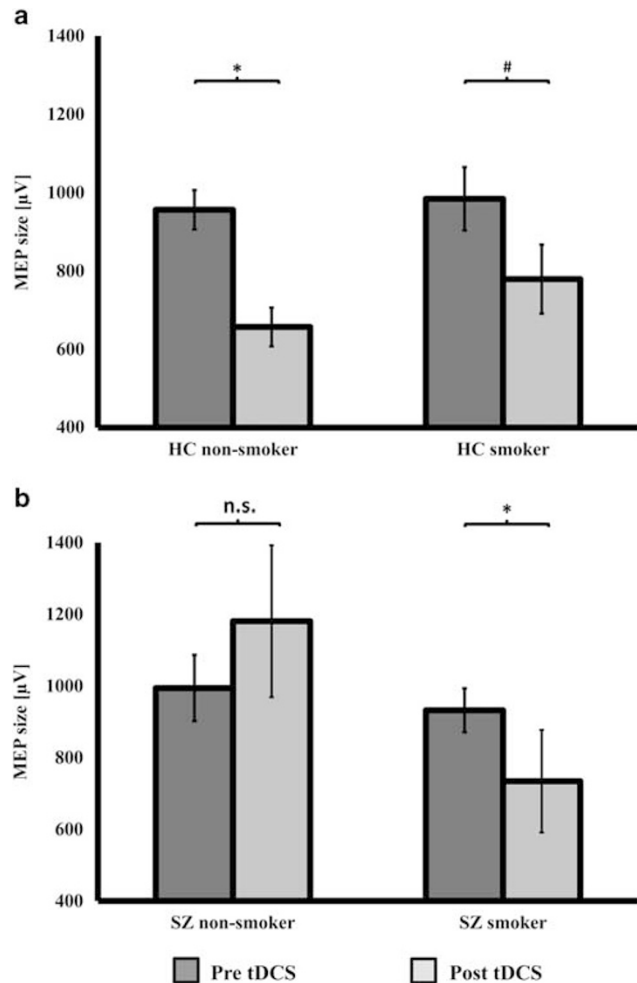05$), whereas the comparison between healthy and schizophrenic smokers showed no significant differences in MEP size ($t_{(28)} = 0.962$, $p = 0.344$) following cathodal tDCS.

Other Parameters of Cortical Excitability before and after Cathodal tDCS

For all other dependent variables, RM-ANOVAs showed no significant main effects or interactions and no further analyses were conducted.

Correlation Analysis

Pearson correlations conducted in the schizophrenic nonsmoking group between dependent variables clinical variables, and CPZ equivalents revealed significant positive correlations between PANSS$_{Negative}$ and post RMT values ($p = 0.037$), and between GAF values and post aMEP values ($p = 0.039$). Furthermore, a negative correlation between PANSS$_{Negative}$ and post-MEP sizes ($p = 0.019$) was revealed in this group. Analyses in the schizophrenia smoking group showed a significant positive correlation between PANSS$_{Negative}$ and post-MEP sizes ($p = 0.012$; Figure 2). In healthy smokers, we found a significant negative correlation between Fagerstroem values and CSP duration ($p = 0.009$), and in schizophrenic smokers, positive correlations between Fagerstroem values and pre-tDCS aMEP ($p = 0.020$), as well as post-tDCS aMEP ($p = 0.018$).

DISCUSSION

Our results provide first evidence for a smoking-induced restitution of impaired LTD-like neuroplasticity following
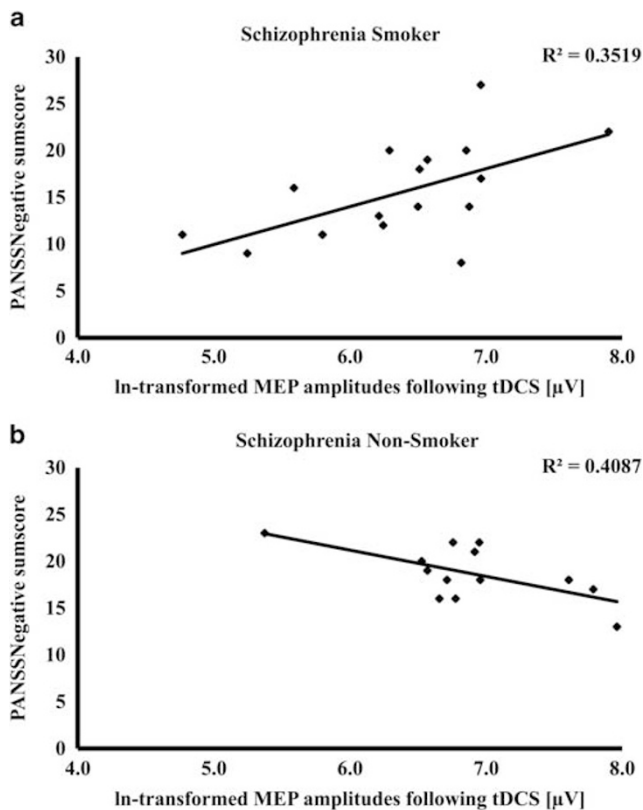

Figure 2 Correlations between the post-MEP values and negative symptoms. Smoking schizophrenia patients (a) showed a significant positive correlation between PANSS$_{Negative}$ and post-MEP sizes ($p = 0.012$), whereas nonsmoking schizophrenia patients (b) showed a negative correlation between PANSS$_{Negative}$ and post-MEP sizes ($p = 0.019$).

cathodal tDCS in schizophrenia patients. Nonsmoking schizophrenia patients displayed abolished LTD-like plasticity compared with both smoking schizophrenia patients and respective healthy controls. In smoking schizophrenia patients, these plasticity deficits were not present. These findings implicate a beneficial effect of nicotine on impaired neuroplasticity in schizophrenia and may contribute to the excessive smoking rates in this population.

Smoking Effect on Cortical Excitability

Foregoing investigations of motor-cortex excitability with TMS have shown a reduction in intracortical facilitation, prolonged CSP durations, enhanced short-afferent inhibition (SAI), and reduced aMEPs, indicating cortical hyopexcitability in chronic healthy smokers during nicotine withdrawal (Grundey *et al*, 2013; Lang *et al*, 2008). However, the duration of nicotine withdrawal differed between the studies, ranging from one (Lang *et al*, 2008) to six (Grundey *et al*, 2013) hours. Our baseline analyses showed no significant differences across all four study groups for RMT, CSP, and aMEPs, but the comparability to the foregoing studies is reduced, as we did not investigate paired-pulse TMS paradigms or short-afferent inhibition in all the participants. Therefore, despite being in the time range of nicotine withdrawal (at least half an hour in our sample), we could not replicate the CSP and aMEP findings described in the respective foregoing publication (Lang *et al*, 2008). Interestingly, we found a significant negative correlation between Fagerstroem values and CSP duration in healthy smokers, indicating at least a gradual impact of smoking on CSP duration. Reviewing the literature for motor-cortical excitability in schizophrenia (Bunse *et al*, 2014), general differences between schizophrenia patients and healthy controls for CSP are subject to a large inter-study variability, possibly influenced by the smoking status of the study subjects, which is usually not reported in these studies. Prolonged, shortened, or unchanged CSP durations in schizophrenia patients were explained by different disease states and different medications (Bunse *et al*, 2014).

Smoking Effect on tDCS-Induced Neuroplasticity in Healthy Subjects

In our study, healthy nonsmokers displayed a significant reduction in MEP amplitudes following cathodal tDCS. In smokers, only a trend-level MEP reduction was obtained. Independent *t*-tests did not show significant differences between healthy smokers and nonsmokers for MEP amplitudes before and after stimulation. These findings are in line with data previously reported for healthy nonsmokers, which displayed a significant MEP reduction following cathodal tDCS in the respective placebo conditions of these drug trials (Grundey *et al*, 2012a; Thirugnanasambandam *et al*, 2011). In the intervention groups receiving either prolonged nicotine patch application (Thirugnanasambandam *et al*, 2011) or fast-acting nicotine nasal spray application (Grundey *et al*, 2012a), the results of these studies showed a reduced or abolished LTD-like neuroplasticity response following cathodal tDCS.

With regard to our findings in healthy smokers, one comparable study showed a significant MEP reduction

following cathodal tDCS in healthy subjects under nicotine withdrawal for 10 h, but only a trendwise MEP reduction following nicotine patch application (Grundey et al, 2012b). As in our experimental setup, only a short period of nicotine withdrawal preceded the experiments and these subjects displayed related LTD-like plasticity responses, as did healthy subjects following prolonged nicotine patch application (Grundey et al, 2012b), so one might argue that our withdrawal duration may be insufficient to restore nicotine-related abolishment of plasticity. The effects in smokers might therefore be at least partially determined by an impact of nicotine. These pharmacological challenges in healthy smokers and nonsmokers suggest that, dependent on pharmacokinetic properties and the number and density of cerebral nAChR (Mukhin et al, 2008), nicotine has a complex but prominent impact on cortical plasticity

Nicotine-Induced Plasticity Restoration in Patients with Schizophrenia

In nonsmoking schizophrenia patients, cathodal tDCS failed to induce LTD-like plasticity, which is consistent with previously described dysfunctional neuroplasticity in schizophrenia patients following tDCS and other noninvasive brain stimulation techniques (Hasan et al, 2013a). In contrast, smoking schizophrenia patients showed a clear LTD-like plasticity response that was even similar to that observed in the respective healthy controls. This questions the current knowledge regarding general plasticity deficits in schizophrenia patients following any kind of noninvasive brain stimulation (Hasan et al, 2013a) by proposing that LTD-like plasticity can also be induced in schizophrenia patients depending on their smoking status.

The interpretation of these novel findings is complex because various underlying mechanisms may contribute to plasticity deficits in schizophrenia. Apart from the effect of the disease itself, foregoing studies have identified antipsychotic treatment as a major contributor to reduced cortical excitability in schizophrenia patients (Liu et al, 2009; Daskalakis et al, 2008b) and one could only speculate in which way and to what extent chronic smoking interacts with different transmitter systems. Our findings may not only be explained by mechanisms involving pre- and postsynaptic cholinergic, glutamatergic and GABAergic neurotransmission leading to complex interactions between inhibition and facilitation of pyramidal neurons (Alkondon et al, 2000; Dani, 2001), but also by structural brain changes. Chronic smoking induces long-lasting modifications of cortical architecture affecting dendritic arborization and nicotinic receptor density (Breese et al, 1997; Gonzalez et al, 2005).

Different lines of evidence contribute to the concept of disturbed neuroplasticity in schizophrenia. Both hypo- and hyperfunction of glutamatergic N-methyl-d-Aspartate (NMDA) receptors have been supposed to result in impaired neuroplasticity (Paz et al, 2008). This glutamatergic dysfunction has also been linked to alterations in GABAergic neurotransmission, as a reduced density of GABAergic interneurons that express certain NMDAR subunits has been shown in neuropathological investigations (Woo et al, 2004). Reduced inhibition in schizophrenia has been linked to a substantial decrease in GABAergic interneurons, the

GABA-synthesizing enzyme glutamic acid decarboxylase (GAD67), and GABA-related gene expression in various parts of the schizophrenia brain (Benes, 2011; Hashimoto et al, 2008b). Adding to the complexity of neuroplasticity regulation, the transmitter release and receptor expression of NMDA and GABA have both been shown to be modulated through cholinergic presynaptic projections (Wang et al, 2007) that increase GABAergic input towards pyramidal cells (McGehee, 2007). Given the influence of nicotine and acetylcholine on the regulation of cortical excitability functions (Alkondon et al, 2000), one could assume that the associations between impaired glutamatergic, GABAergic, and cholinergic neurotransmission might result in an imbalance between facilitation and inhibition tilted toward disinhibition and excessive excitatory pruning, as discussed as one important pathophysiological feature of schizophrenia (Rapoport et al, 2012).

Alternatively, assuming a neuronal effect of nicotine after half an hour withdrawal, the impact of nicotine on calcium influx via $\alpha 7$- and $\alpha 4\beta 2$ receptors together with tDCS-induced NMDA-receptor activation would shift plasticity to the so-called 'no mans land' (Lisman, 2001) in healthy smokers. In nonsmoking schizophrenia patients, one could speculate that the well-established NMDA-receptor hypoactivity (Javitt et al, 2012) would not be sufficient to induce LTD-like plasticity, but a nicotine-induced increase in calcium, as outlined above, would add sufficient calcium to induce LTD-like neuroplasticity in smoking schizophrenia patients.

Functional Implications

Concerning the hereby-entailed consequences for neuroplasticity in patients with schizophrenia, different functional consequences have to be taken into account. A reduced signal-to-noise ratio with a consecutive reduced filter-function has been proposed to explain cognitive and memory deficits in schizophrenia (Rolls et al, 2008). Reviewing the available literature for cortical excitability and plasticity in schizophrenia using noninvasive brain stimulation, a clear picture emerges. Reduced cortical inhibition (eg, reduced short-interval intracortical inhibition, for review see (Bunse et al, 2014)) might cause enhanced cortical noise, leading to reduced spike-timing-dependent plasticity (as measured by paired-associative stimulation) (Frantseva et al, 2008) and reduced nonfocal plasticity (as measured by anodal and cathodal tDCS) via enhanced asynchronous baseline cortical activity (Hasan et al, 2012b; Hasan et al, 2011)

Our observed effect of enhanced LTD-like plasticity associated with nicotine intake through chronic smoking might reduce cortical noise and thus increase the signal-to-noise ratio in schizophrenia patients. This would be in line with the observation that LTD has an important role in hippocampal-based learning and memory functions (Collingridge et al, 2010), suggesting a direct contribution of LTD to information storage (Kemp and Manahan-Vaughan, 2007). In accordance, a noise-reducing effect of cathodal tDCS was suggested in previous experiments (Antal et al, 2004). Evidence from functional imaging (Winterer et al, 2006), as well as from neurophysiological studies (Hasan et al, 2013a), has linked the concept of a

reduced signal-to-noise ratio to the observation of prefrontal microcircuitry dysfunction in patients with schizophrenia, which is thought to be of key relevance for attention and working memory deficits, as both cognitive processes appear to be conveyed in particular by the prefrontal cortex (PFC; Levin, 1992). Regarding the involvement of the cholinergic system and the potential influence of nicotine, evidence points to the notion that neuroplasticity in the PFC is mediated substantially through nAChR activity (McGehee, 2007). Furthermore, it was shown that nicotine intake can improve the cortical signal-to-noise ratio by enhancing GABAergic neurotransmission (Couey et al, 2007).

Thus, chronic smoking might enhance the signal-to-noise ratio via restitution of LTD-like neuroplasticity by enhancing GABAergic neurotransmission or calcium influx in schizophrenia patients and thereby improve functions. Interestingly, the smoking schizophrenia patients had less negative symptoms compared with the nonsmoking patients. Moreover, in smoking schizophrenia patients, negative symptoms correlated positively with post-tDCS MEP sizes, whereas nonsmoking schizophrenia patients showed the opposite effect. This indicates that enhanced LTD-like plasticity in smoking schizophrenia patients is related to reduced negative symptoms.

Limitations

For a comprehensive interpretation of our results, some important limitations have to be taken into account. The first limitation of this study relates to the experimental design, which had to be adapted to the procedure tolerance of schizophrenia patients and thus is shorter than investigations in healthy controls. Furthermore, data of a comparative sample receiving sham tDCS were not available to correct for a putative bias related to regression to the mean in the schizophrenia group following stimulation. Second, we did not recruit patients consecutively, but integrated data from three projects (Hasan et al, 2012a; Hasan et al, 2013b; Hasan et al, 2012b) to answer this specific research question. Third, we neither tested anodal tDCS nor different PAS protocols, which would be important to specifically address the functional consequences of chronic smoking with regard to the focusing effect of nicotine. The fourth limitation is that the duration of nicotine withdrawal of at least half an hour in our study differed from the 10 h in some foregoing study protocols. Hence, the comparability of our data for healthy subjects to previous research is restricted. As we cannot rule out differences in the timing of the last cigarette between smoking schizophrenia and healthy subjects and as the degree of nicotine dependence is different between these groups, both factors have to be considered as potential confounding variables. One should also note that many different compounds in tobacco smoke aside from nicotine could account for some of the observed effects. As no pharmacological administration of pure nicotine was performed, conclusions as to what extent other tobacco smoke compounds might contribute to neuroplasticity remain elusive. Future studies need to clarify the effects of nicotine on cortical plasticity in schizophrenia using, eg, nicotine transdermal patches agents or nasal spray.

Furthermore, we did not obtain blood levels or breath CO concentration to verify compliance of smoking subjects. A further limitation is that schizophrenia patients were on a stable antipsychotic treatment and we cannot rule out an impact of antipsychotics on cortical plasticity or specifically test the interaction between antipsychotics, smoking, and cortical plasticity. Finally, though being within the range of other studies in the field, our sample size is rather small, meaning that some results would not survive correction for multiple testing.

Summary and Outlook

Our study shows that chronic smoking restores reduced LTD-like plasticity following cathodal tDCS in schizophrenia patients and provides a new biological explanation for the increased rates of nicotine dependency in this population. Furthermore, our findings contribute to the notion that nicotine intake through smoking might stabilize the impaired inhibition–facilitation balance in the schizophrenic brain and thus helps to increase a pathologically reduced signal-to-noise ratio. These network effects might be relevant for reducing negative symptoms and could explain the cognitive enhancing effect of smoking in schizophrenia. As the effects of smoking on cortical plasticity were not taken into account in the past, future studies need to investigate the impact of nicotine to allow an in-depth understanding of impaired neuroplasticity in schizophrenia. With regard to the future development of nicotinergic drugs, our study delivers additional support for an important role of nicotinergic stimulation for the treatment of schizophrenia.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)