

# Smoking status ameliorates cholinergic impairments in cortical inhibition in patients with schizophrenia

Benjamin Pross<sup>a,1,\*</sup>, Susanne Muenz<sup>b,1</sup>, Michael A. Nitsche<sup>c</sup>, Frank Padberg<sup>b</sup>,  
Wolfgang Strube<sup>a</sup>, Irina Papazova<sup>a</sup>, Peter Falkai<sup>b</sup>, Alkomiet Hasan<sup>a</sup>

<sup>a</sup> Department of Psychiatry, Psychotherapy, and Psychosomatic Medicine, Faculty of Medicine, University of Augsburg, Bezirkskrankenhaus Augsburg, Geschwister-Schönert-Str. 1, 86156 Augsburg, Germany

<sup>b</sup> Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany

<sup>c</sup> Leibniz Research Centre for Working Environment and Human Factors at TU Dortmund, Dept. Psychology and Neurosciences, Germany

## 1. Introduction

Schizophrenia is a severe psychiatric disorder associated with life-style characteristics such as significantly increased smoking rates (60–90%; Chapman et al., 2009; de Leon and Diaz, 2005; Dome et al., 2010). Various links between the disorder and smoking are discussed, but knowledge about neurophysiological effects, and in particular cortical excitability changes, of chronic nicotine consumption in patients with schizophrenia is lacking.

The main neuroactive component of smoking – nicotine – binds to nicotinic acetylcholine receptors (nAChR), ligand-gated ion channels

which increase neuronal calcium permeability, thereby affecting cortical excitability and neuroplasticity (Dani and Bertrand, 2007). Since nAChR are often located non- or presynaptically, nicotine has complex modulating effects on other neurotransmitters such as glutamate, dopamine and GABA, which are not easily translatable into functional effects (Dajas-Bailador and Wonnacott, 2004; Descarries et al., 1997; McGehee et al., 1995; Sher et al., 2004; Zarei et al., 1999).

On a functional level, nicotine has been reported to affect cognition and behavior. Acute nicotine administration improved cognitive performance both in healthy subjects and in patients with schizophrenia (Hahn et al., 2013; Wing et al., 2013). Chronic nicotine consumption, on

---

\* Corresponding author.

E-mail address: [benjamin.pross@med.uni-augsburg.de](mailto:benjamin.pross@med.uni-augsburg.de) (B. Pross).

<sup>1</sup> Both authors contributed equally.

the other hand, leads to a complex pattern of up- and downregulation of nAChR (Even et al., 2008), and has been associated with cognitive impairment, both in healthy subjects (Anstey et al., 2007; Sabia et al., 2008) as well as schizophrenia patients (Zhang et al., 2012). However, chronic smokers with schizophrenia report reduced severity of negative symptoms (Zhang et al., 2012). Moreover, upregulation of nAChR in chronic smokers with schizophrenia has been associated with reduced negative symptoms and improved cognitive performance (Esterlis et al., 2014). Therefore, it has been suggested that the high smoking rate among patients with schizophrenia reflects an attempt to ameliorate existing symptoms (Winterer, 2010; but see also Dome et al., 2010). In that regard, add-on application of  $\alpha 7$ -nAChR agonists to ongoing antipsychotic treatment in patients with schizophrenia was not effective in terms of cognitive functions, but showed subtle effects on negative symptoms as shown in a recent meta-analysis of 13 trials (Recio-Barbero et al., 2021).

A potential mediator of nicotinic effects on cognition and behavior might be its effect on cortical excitability (Grundey et al., 2018b). However, knowledge about the neurophysiological basis of these effects is limited so far, especially in patient populations.

To shed light on potential mechanisms of smoking-induced nicotinic effects on cortical excitability in schizophrenia, we first describe neurophysiological alterations associated with the disease. In particular, transcranial magnetic stimulation (TMS) protocols assessing cortical excitability have suggested deficits in cortical inhibition.

Decreased short interval intracortical inhibition (SICI) was reported in the majority of studies in medicated, unmedicated, first-episode and chronic schizophrenia patients (Bunse et al., 2014; Hasan et al., 2011; Radhu et al., 2013). These results imply impairments of GABAergic neurotransmission in patients with schizophrenia (di Hou et al., 2021).

By contrast, intracortical facilitation (ICF), which mainly reflects glutamatergic activity and is modulated by NMDA and GABA-A receptors, showed no clear alterations in schizophrenia (Bunse et al., 2014; Radhu et al., 2013).

Short-latency afferent inhibition (SAI), a paired-pulse TMS marker of cholinergically driven cortical inhibition, is reduced in patients with schizophrenia compared to healthy subjects, as suggested by two studies so far. In a small-sample study ( $N = 10$ ), Shoyama et al. (2013) reported reduced SAI in the motor cortex, while Noda et al. (2017;  $N = 24$ ) discovered reduced SAI in frontal areas, but not the motor cortex via a TMS-EEG protocol. Reduction of SAI implies impairments of cholinergic inhibitory networks in patients with schizophrenia. This is in line with a growing body of evidence implicating a dysregulation of the cholinergic system in the pathophysiology of schizophrenia (D'Souza and Markou, 2012), including alterations in acetylcholine (ACh) levels (Bustillo et al., 2002; Théberge et al., 2004), number of ACh neurons (Garcia-Rill et al., 1995), as well as nAChR expression and function (Breese et al., 2000; Durany et al., 2000; Guan et al., 1999). Polymorphisms of nAChR genes have also been linked to this disease (Mathew et al., 2007). On a functional level, a lower volume of ACh regions was associated with cognitive impairments in patients with schizophrenia (Avram et al., 2021), and nicotine as well as other nAChR agonists have been shown to improve cognition in patients with schizophrenia (Freedman, 2014; Terry and Callahan, 2020).

Up to now, the effects of smoking on cortical excitability have not been studied in patients with schizophrenia. Studies in healthy subjects showed increased cortical inhibition as well as reduced facilitation in chronic smokers compared to non-smokers. ICF was reduced in smokers during nicotine withdrawal, arguing for reduced presynaptic release of glutamate, potentially as a result of a presynaptic nAChR downregulation in chronic smokers, while SICI did not differ between groups (Grundey et al., 2013; Lang et al., 2008).

Most importantly, chronic healthy smokers showed a larger amount of inhibition, as measured by SAI compared to non-smoking individuals (Grundey et al., 2013; Lang et al., 2008), probably due to nicotine-driven changes of cholinergic circuits. Moreover, corticospinal

excitability (input/output (I/O) curve) was increased in smokers (Grundey et al., 2013; Khedr et al., 2020) although this result was not shown by Lang et al. (2008) who observed no differences in the I/O curve. Other measures of corticospinal excitability, such as resting motor threshold (RMT), and the TMS intensity required to obtain a 1 mV peak-to-peak amplitude ( $SI_{1mV}$ ) did not show differences between healthy smokers and non-smokers (Grundey et al., 2013; Khedr et al., 2020; Lang et al., 2008).

The increased cortical inhibition observed in healthy smokers suggests that smoking may have a restitution effect on decreased inhibition reported in patients with schizophrenia (Shoyama et al., 2013). Modulation of cortical excitability through nicotine is assumed to have furthermore a direct impact on the behavioral level (Grundey et al., 2018a; Grundey et al., 2018b) and might be an important neurophysiological basis for its cognitive effects, which we see in both healthy subjects and patients with schizophrenia.

In the present study we aimed to evaluate several parameters of cortical excitability in smoking and non-smoking schizophrenia patients using various established TMS protocols. We assumed that excitability measures would differ depending on smoking status, more specifically, that patterns of reduced cortical inhibition associated with schizophrenia would be normalized in smokers. Following the results in healthy subjects, we expected more inhibitory effects of various TMS protocols in subjects with schizophrenia for smokers under withdrawal compared to non-smokers.

## 2. Methods

### 2.1. Subjects

Twenty-six smokers and 19 non-smokers diagnosed with schizophrenia according to ICD-10 were recruited from local inpatient and outpatient units of the psychiatric LMU hospital. Exclusion criteria were a history of epilepsy, brain injuries or tumors, metal implants in the head, current alcohol or drug dependence (other than nicotine), current intake of antidepressants or anticonvulsants, regular intake of benzodiazepines, age under 18 or over 65, and pregnancy. Based on previous studies (Grundey et al., 2013; Lang et al., 2008), we classified participants as smokers if they had consumed 10 or more cigarettes per day within the past five years and scored at least 3 points on the Fagerstroem Test for Nicotine Dependence (indicating moderate nicotine dependence). Non-smokers were defined as having no history of nicotine consumption within the past five years. Besides the Fagerstroem Test, smoking status was verified by measuring breath carbon monoxide (CO). All smokers were asked to abstain from nicotine for at least 8 h before the experiment, which was checked with a second breath CO measurement directly before the start of the experiment. A substantial reduction of the CO level had to be evident in order to be considered as abstinent. Patients underwent an assessment of psychopathology (Positive and Negative Syndrome Scale, PANSS; Calgary Depression Scale for Schizophrenia, CDSS), disease severity (Clinical Global Impression, CGI) and social functioning (Global Assessment of Functioning, GAF). Handedness was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). All but one patient were treated with antipsychotics (18 in monotherapy, 26 in combination therapy). Chlorpromazine equivalents of daily doses were calculated according to Leucht et al. (2016). All volunteers gave their written informed consent before participation. The study was conducted in accordance with the Declaration of Helsinki and approved by the medical faculty of the LMU Munich (approval number: 17-280).

Demographic and clinical characteristics of the participants are shown in Table 1.

### 2.2. Application of transcranial magnetic stimulation (TMS)

We used single- and paired-pulse TMS protocols to monitor standard

**Table 1**

Demographic and clinical characteristics of the subjects.

Variable	Smokers	Non-smokers	$\chi^2$ -/t-value	p-value
N	26	19	–	–
Gender	17 M, 9F	10 M, 9F	0.744 <sup>a</sup>	0.388
Age (y)	34.6 ± 13.4	38.7 ± 7.3	1.317 <sup>b</sup>	0.345
Handedness	23 R, 2 A, 1 L	16 R, 3 L	2.775 <sup>a</sup>	0.337
Fagerstroem score	5.2 ± 1.6			
CO (ppm) Baseline	20.2 ± 11.4	2.5 ± 1.5	-6.747 <sup>b</sup>	< 0.001*
CO (ppm) during experiment	11.5 ± 8.1		-4.324 <sup>c</sup>	< 0.001*
PANSS scores				
Total	53.6 ± 11.8	52.1 ± 11.6	-0.442 <sup>b</sup>	0.661
Positive	12.2 ± 4.1	12.1 ± 4.0	-0.082 <sup>b</sup>	0.936
Negative	14.9 ± 5.1	13.6 ± 5.3	-0.859 <sup>b</sup>	0.395
General	25.5 ± 6.6	26.4 ± 5.8	0.464 <sup>b</sup>	0.645
CDSS	2.9 ± 2.4	2.8 ± 2.8	-0.121 <sup>b</sup>	0.904
GAF	63.6 ± 9.4	67.7 ± 10.6	1.361 <sup>b</sup>	0.181
CGI	3.5 ± 0.6	3.3 ± 0.6	-0.830 <sup>b</sup>	0.411
CPZ (daily)	485.8 ± 293.9	390.0 ± 259.2	-1.135 <sup>b</sup>	0.263
Antipsychotic medication			1.505 <sup>a</sup>	0.529
Monotherapy	10	8	–	–
Combination treatment	16	10	–	–
None	0	1	–	–
Duration of psychosis (y)	7.3 ± 7.7	11.9 ± 9.4	1.823 <sup>b</sup>	0.075

Abbreviations: CO (ppm), Carbon monoxide concentration in parts per million measured from breath carbon monoxide; PANSS, Positive and Negative Syndrome Scale; CDSS, Calgary Depression Scale for Schizophrenia; GAF, Global Assessment of Functioning; CGI, Clinical Global Impression; CPZ, chlorpromazine equivalent dose.

Data are presented as mean ± standard deviation.

\* Test was significant at  $\alpha = 0.05$ . For details, see text.

<sup>a</sup> Chi-square test.

<sup>b</sup> Unpaired *t*-test.

<sup>c</sup> Paired *t*-test smokers CO Baseline compared to smokers CO during experiment.

parameters of corticospinal and intracortical excitability. TMS was delivered with a MagStim BiStim<sup>2</sup> (The Magstim Co. Ltd, Whitland, UK) using a standard figure-of-eight alpha coil (70 mm diameter) at a frequency of 0.2 Hz. The coil was positioned over the left primary motor cortex in both right- and left-handed subjects, at the site where stimulation consistently induced the largest and most stable motor-evoked potentials (MEPs) in the contralateral right first dorsal interosseous muscle (FDI). According to standard practice, the coil was held tangentially to the head at a 45° angle.

### 2.3. Electromyography recording

Surface electromyographic (EMG) activity was recorded from the right FDI with two Ag-AgCl surface electrodes in a belly-tendon montage. Raw signals were amplified with a gain of 1000, bandpass-filtered (2 Hz–3 kHz); D-360, Digitimer Ltd., Welwyn Garden City, UK) and digitized at 5 kHz using a CED 1401 data acquisition interface controlled by Signal software v5.08 (Cambridge Electronic Design Ltd., Cambridge, UK). Data were stored digitally and analyzed offline using the Signal Software and NuCursor (J.C. Rothwell, Institute of Neurology, University College London, UK).

### 2.4. Single-pulse measures

Two TMS intensities served as the basis for all protocols: the stimulation intensity needed to elicit MEPs of approximately 1 mV peak-to-peak amplitude ( $SI_{1mV}$ , expressed as percentage of maximal stimulator output), as well as the resting motor threshold (RMT). RMT was defined

as the minimal intensity that produced stable MEPs with an amplitude of approximately 50  $\mu$ V in at least 5 out of 10 consecutive trials (Rossini et al., 2015).

#### 2.4.1. Input-output curve (I/O curve)

The I/O curve was obtained using TMS intensities of 90, 110 and 130 % RMT. Blocks of 10 pulses for each intensity were recorded consecutively, starting with the lowest intensity.

### 2.5. Paired-pulse measures

#### 2.5.1. Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF)

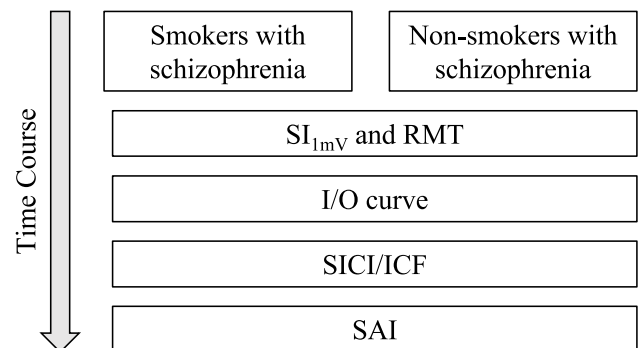
SICI and ICF were recorded with a paired-pulse protocol (Kujirai et al., 1993), with interstimulus intervals (ISI) of 2, 3, 7, 9 and 12 ms. The intensity of the conditioning and test pulses was set to 80 % RMT and  $SI_{1mV}$ , respectively. 15 trials for each ISI as well as 25 control trials with the test pulse alone were administered in randomized order.

#### 2.5.2. Short-latency afferent inhibition (SAI)

SAI was obtained by pairing a conditioning peripheral nerve stimulus with a subsequent TMS stimulus, with ISIs of 20 ms and 40 ms (for review see: Turco et al. 2018). Peripheral stimulation (square pulse, width 0.2 ms) was applied to the right ulnar nerve at wrist level using a Digitimer DS7A stimulator (Digitimer Ltd., UK) with a bipolar electrode (cathode positioned proximally). The stimulation intensity was set to 200% of the perceptual threshold ( $4.47 \pm 2.00$  mA), which was determined by increasing the stimulation intensity incrementally from a subthreshold level until the participant reported a sensation. The lowest intensity reliably perceived by the participant was defined as the perceptual threshold. TMS intensity was set to  $SI_{1mV}$ . 20 paired pulses for each ISI and 20 control pulses (single TMS pulse) were administered in random order.

### 2.6. Experimental procedures

The participants were seated in a comfortable chair with head and arm rests. First, EMG electrodes were placed over the right FDI and TMS was applied to the left primary motor cortex to identify the optimal coil position for inducing MEPs in the right FDI, as described above. The position was marked with a pen to ensure that it remained consistent throughout the experiment. After determining  $SI_{1mV}$  and RMT, the protocols for I/O curve, SICI/ICF and SAI were administered in that order (see Fig. 1).



**Fig. 1.** Experimental course of the study. Smokers and non-smokers underwent TMS over the left primary motor cortex, eliciting MEPs in the right FDI. First,  $SI_{1mV}$  and RMT were determined, then I/O curve, SICI/ICF and SAI protocols were measured.

## 2.7. Data analysis

Statistical analysis was performed using JASP (Version 0.14.1). Before further analyses, we manually inspected the raw data and excluded MEPs if 1) MEPs were larger than 5 mV, 2) patient movement artefacts were apparent or 3) there was a pre-contraction in the target muscle. In total 17 MEPs were removed which corresponds to 0.2% of all MEPs recorded. Next, boxplots were used to identify outliers (values more than 1.5x the interquartile range above/below the upper/lower quartile) in the normalized mean MEPs (see below) for each protocol condition. All data from the corresponding participants were excluded from analysis of the respective protocols (I/O curve: 3 smokers, 3 non-smokers; SICI/ICF: 3 smokers, 2 non-smokers; SAI: 1 smoker, 2 non-smokers).

Group differences in the demographic and clinical data were evaluated with two-sided unpaired t-tests and chi-square tests. To rule out differences in  $SI_{1mV}$  and RMT between the groups unpaired t-tests were performed.

For the I/O curve, we calculated the intraindividual means of MEP amplitudes for each stimulation intensity. These were entered as dependent variable in a mixed-model analysis of variance (ANOVA) with ‘group’ (smokers vs. non-smokers) as between-subject factor and ‘TMS intensity’ (90 vs. 110 vs. 130 % RMT) as within-subject factor.

For the paired-pulse protocols (SAI, SICI/ICF), the intraindividual mean of conditioned MEP amplitudes for each ISI was normalized to the respective single-pulse control condition (expressed in percentage of the control condition) to evaluate the effect of the conditioning stimulus on the MEP amplitude. To confirm that the control conditions did not differ between groups, the mean MEPs of the single-pulse conditions were compared with two-sided unpaired t-tests. To investigate the effects of smoking status on SAI and SICI/ICF, a mixed-model RM-ANOVA was conducted for each protocol using the normalized mean MEPs as dependent variable, ‘group’ (smokers vs. non-smokers) as between-subject factor and ‘ISI’ (SAI: 20 vs. 40 ms; SICI/ICF: 2 vs. 3 vs. 7 vs. 9 vs. 12 ms) as within-subject factor. If necessary, we performed Mauchly’s test of sphericity and used the Greenhouse-Geisser correction. In cases of significant effects from the mixed-model RM-ANOVAs, exploratory post-hoc analyses were conducted as detailed in the results session (see 3.5). The critical p-value chosen for significance was 0.05 for all statistical analyses.

## 3. Results

### 3.1. Sociodemographic and clinical characteristics

The groups did not differ significantly regarding gender, age, handedness, clinical scores or medication (see Table 1). On average, patients had mild to moderate positive and negative symptoms (PANSS), a mild to moderate degree of illness (CGI) and mild impairment of functioning (GAF). The mean Fagerstroem score in the smoking group ( $5.2 \pm 1.6$ ) indicated strong nicotine dependence, which was underlined by a mean breath carbon monoxide level of  $20.2 \pm 11.4$  ppm. TMS was well tolerated, and no adverse events were reported. Some patients reported tolerable symptoms of withdrawal such as craving and restlessness.

### 3.2. Stimulation parameters

Mean stimulation parameters are presented in Table 2. The TMS intensities required to evoke MEPs of 1 mV amplitude did not differ significantly between groups ( $t[43] = -0.983, p = 0.331$ ), nor did the RMT ( $t[43] = -1.002, p = 0.322$ ).

### 3.3. I/O curve

For the I/O curve, the mixed-model RM-ANOVA revealed the expected main effect of the factor ‘TMS intensity’ ( $F[2, 74] = 70.634, p <$

**Table 2**

Mean values of stimulus intensities required to elicit MEPs of 1 mV amplitude and resting motor threshold for smokers and non-smokers.

	smokers <i>M ± SD</i>	non-smokers <i>M ± SD</i>	<i>t</i> -value	<i>p</i> -value
$SI_{1mV}$	47 ± 12	43 ± 11	-0.983	0.331
RMT	38 ± 10	35 ± 9	-1.002	0.332

Abbreviations:  $SI_{1mV}$ , TMS intensity required to elicit MEPs of 1 mV amplitude, expressed as percentage of maximal stimulator output; RMT, resting motor threshold; M, mean; SD, standard deviation.

Unpaired t-tests did not reveal significant differences between groups for both parameters.

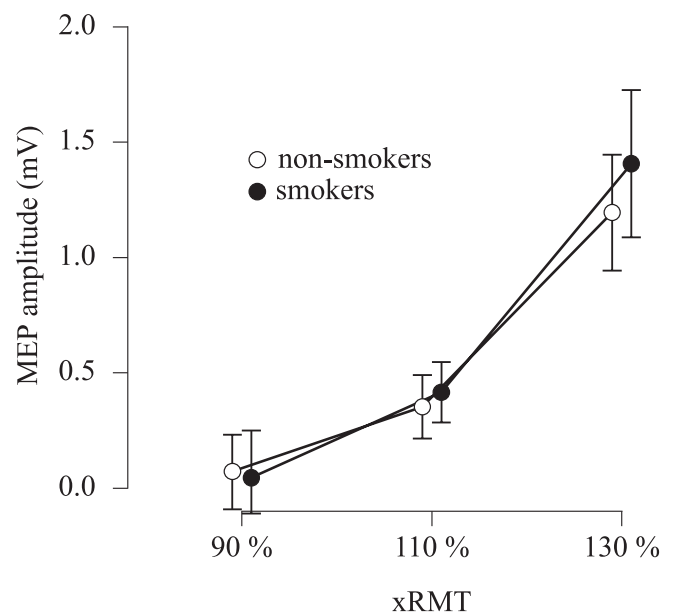
0.001), but no effect of the between-subject factor ‘group’ ( $F[1, 37] = 0.494, p = 0.548$ ), nor an interaction effect ( $F[2, 74] = 0.620, p = 0.541$ ) (see Fig. 2). Chronic smoking did not have a significant impact on corticospinal excitability measured with the I/O curve.

### 3.4. SICI/ICF

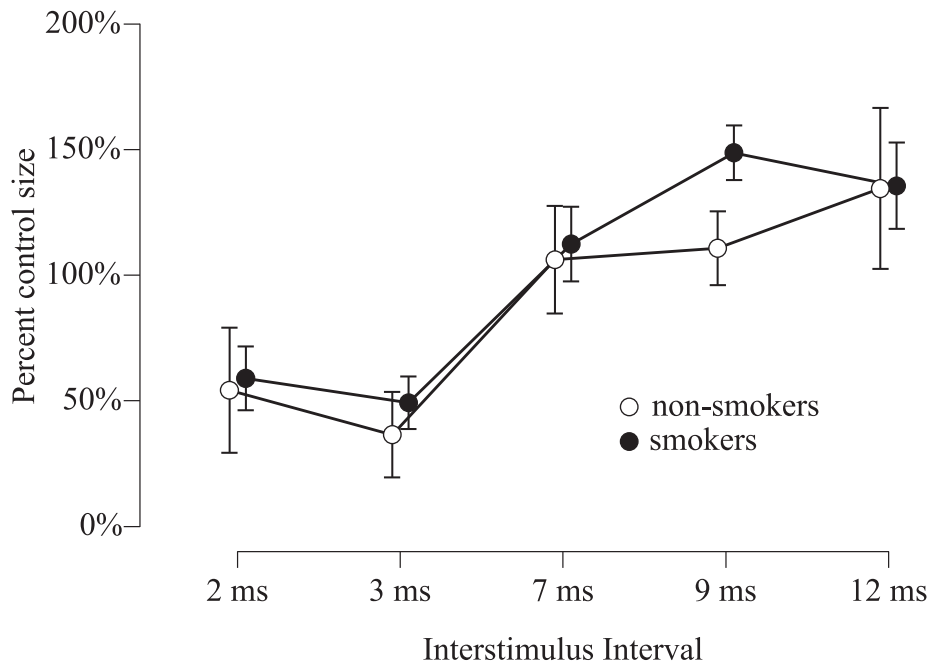
Single-pulse MEPs (test pulse) differed between groups ( $t[38] = 2.351, p = 0.024$ ). The mixed-model RM-ANOVA showed a significant effect of the factor ‘ISI’ ( $F[2.306, 87.637] = 51.629, p < 0.001$ ), but no significance of the factor ‘group’ ( $F[1, 38] = 1.559, p = 0.220$ ) or the interaction between these factors ( $F[2.306, 87.637] = 1.578, p = 0.209$ ) (see Fig. 3). The results showed thus no significant impact of chronic nicotine consumption on intracortical inhibition or facilitation.

### 3.5. SAI

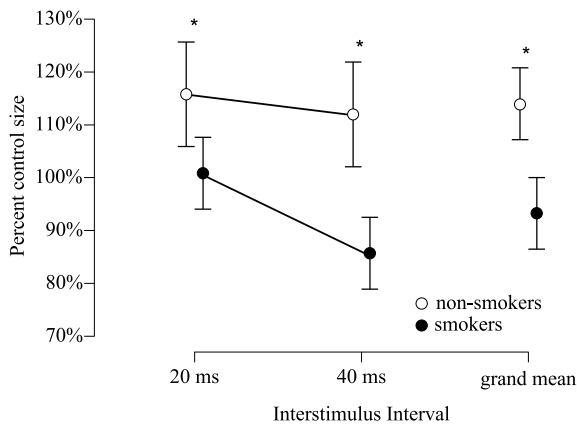
Single-pulse MEPs (test pulse) did not differ between groups ( $t[40] = -1.779, p = 0.083$ ). For the SAI protocol, the mixed-model RM-ANOVA showed a significant effect of the factor ‘ISI’ ( $F[1, 40] = 4.381, p = 0.043$ ), and a significant effect of the factor ‘group’ ( $F[1, 40] = 12.023, p = 0.001$ ), but no significant interaction between these factors ( $F[1, 40] = 1.569, p = 0.218$ ) (see Fig. 4). Smoking participants showed a significantly stronger inhibition in the SAI protocol compared to non-smokers.



**Fig. 2.** Input-output curve (I/O curve) values in non-smokers and smokers with schizophrenia. Shown are the mean average values for each intensity. xRMT stands for the percentage of motor threshold used for the TMS intensity. The values are shown as mean ± 95% CI. The smoking status in schizophrenia patients did not lead to significant differences in I/O curve values.



**Fig. 3.** Short-latency intracortical inhibition and facilitation (SICI/ICF) was tested with paired-pulsed motor cortex TMS at different interstimulus intervals of 2–12 ms. The amplitudes are shown as percentage of the amplitudes of the test MEP (single TMS pulse) as mean  $\pm$  95% CI. The mixed-model RM-ANOVA showed no significant difference comparing smokers to non-smokers.



**Fig. 4.** Short-latency afferent inhibition (SAI) in non-smokers and smokers with schizophrenia. The normalised mean values are shown as mean  $\pm$  95% CI. Besides the mean amplitudes evoked by different interstimulus intervals (ISI), the overall mean values are shown. Post hoc analysis revealed significantly larger inhibition in smokers. (\*) represent significant findings ( $p < 0.05$ ).

Exploratory two-sided unpaired  $t$ -tests confirmed this larger inhibitory effects in smokers compared to non-smokers for the 20 ms condition ( $t[40] = 2.424, p = 0.020$ ), the 40 ms condition ( $t[40] = 3.062, p = 0.004$ ) and the grand mean across both conditions ( $t[40] = 1.090, p = 0.001$ ).

With the grand mean MEPs, a one-sample  $t$ -test against 100% was run to determine whether our sample as a whole showed an inhibitory reaction in the SAI protocol. The mean normalized MEP score ( $M = 101.611\%$ ,  $SD = 21.295$ ) was slightly higher than the normalized control condition of 100%. The mean difference of 1.611% was not statistically significant ( $t(41) = 0.490, p = 0.627$ ), implying no inhibitory effect in the overall sample in the SAI protocol.

#### 4. Discussion

This study investigated the neurophysiological changes in motor cortex excitability associated with chronic nicotine consumption in patients with schizophrenia. We compared patterns of excitability measured with TMS in smokers under withdrawal and non-smokers. We expected excitability measures to differ depending on smoking status, more specifically, that schizophrenia-associated patterns of excitability, such as impaired inhibitory processes, might be normalized in smokers.

Contrary to our expectations, intracortical facilitation and inhibition did not differ between smoking and non-smoking patients, nor did corticospinal excitability, as tested with the I/O curve, RMT and  $SI_{1mV}$ . However, SAI results did reveal the expected group differences with larger inhibition in smoking schizophrenia patients. This is in line with our hypothesis that smoking might to some extent normalise schizophrenia-related patterns of excitability, in this case deficient cortical, cholinergically driven inhibition. These results provide evidence for an ameliorating effect of smoking on cortical excitability alterations in schizophrenia.

Regarding corticospinal excitability, the I/O curve results did not reveal significant differences between smokers and non-smokers with schizophrenia. Previous studies with healthy participants reported similar results (Lang et al., 2008). Other studies in healthy subjects showed however increased MEP amplitudes in smokers (Grundey et al., 2013; Khedr et al., 2020), but mainly with TMS intensities above 130% RMT that we used. Thus, the glutamatergic and GABAergic systems (Ziemann et al., 2015) associated with net corticospinal excitability, as measured in the present protocol, do not seem to be impacted relevantly by chronic nicotine consumption in patients with schizophrenia.

Intracortical inhibition, as measured by SICI is known to be impaired in patients with schizophrenia (Bunse et al., 2014; Radhu et al., 2013). Our results show that chronic nicotine consumption does not lead to a significant difference regarding intracortical inhibition in patients with schizophrenia. This is in line with results in healthy subjects showing no differences between smokers and non-smokers for the SICI protocol (Grundey et al., 2013; Lang et al., 2008). GABAergic neurotransmission underlying intracortical inhibitory effect does not seem to be impacted

by chronic nicotine consumption in patients with schizophrenia.

Chronic nicotine consumption did not affect intracortical facilitation measured by the ICF protocol with ISIs of 9 ms and 12 ms. Studies in healthy smokers showed reduced ICF in smokers under withdrawal compared to healthy non-smokers with similar ISIs (Grundey et al., 2013; Lang et al., 2008). Since intracortical facilitation is thought to be controlled predominantly by glutamate (Ziemann et al., 2015), this implies that chronic nicotine consumption does not cause significant differences in the glutamatergic system in patients with schizophrenia.

In the SAI protocol, smokers showed significantly more inhibition compared to non-smokers. Smoking seems to affect the cholinergic mechanisms underlying SAI, leading to an increase in inhibitory activity. In accordance, previous studies comparing healthy smokers with non-smokers showed a larger amount of inhibition in smokers (Grundey et al., 2013; Lang et al., 2008) in the SAI protocol.

The increased cortical inhibition observed in smokers with and without schizophrenia suggests that smoking may have an ameliorating effect on impaired cortical inhibition observed in patients with schizophrenia. The extent to which this neurophysiological effect affects the behavioral level is unclear. It seems tempting to assume that this restoring effect may be related with the decreased negative symptoms in chronic smokers with schizophrenia (Oliveira et al., 2018; Zhang et al., 2012) or improved cognitive functions in smokers with and without schizophrenia (Anstey et al., 2007; Coustals et al., 2020; Durazzo et al., 2012; Paul et al., 2006; Zhang et al., 2012). However, direct evidence to support this assumption is lacking.

In an additional analysis, our sample as a whole showed a lack of physiological inhibition in the SAI protocol. Reduced inhibition in the SAI protocol in patients with schizophrenia compared to healthy subjects was shown already in previous studies with lower sample sizes (Shoyama et al., 2013). These neurophysiological findings suggest that cholinergically driven inhibitory circuits are impaired in patients with schizophrenia. Using different methodological approaches, cholinergic impairments in patients with schizophrenia have been shown in a large number of studies and are even discussed as a trigger for the onset of schizophrenia (Tani et al., 2015).

By ACh agonists, several studies addressed these impairments pharmacologically to explore the effects of ACh substitution at the behavioral level. Indeed, acute effects of ACh agonists were a reduction of positive and negative symptoms (Brannan et al., 2021) and improvements in cognitive functions (Dondé et al., 2020).

#### 4.1. Limitations

Smoking participants abstained from nicotine for 8 h before participating in our experiment. Considering the heavy nicotine dependency of our participants (mean Fagerstrom  $5.2 \pm 1.6$ ), this is a long abstinence and led to reported symptoms of withdrawal such as craving and restlessness. We did not control for the impact of withdrawal itself and can therefore not conclude in how far the results are based on chronic nicotine consumption leading to an upregulation of nAChR (Turner et al., 2011) in combination with a desensitization of nAChR, and to what extent these are the consequences of withdrawal, meaning less nAChR activation. Studies in healthy participants showing lower nicotine dependency (mean Fagerstrom  $2.92 \pm 0.4$  and  $3.19 \pm 0.24$ ) and shorter times of withdrawal (6 h or 1 h) (Grundey et al., 2013; Lang et al., 2008) showed however similar cholinergically driven effects, such as more inhibition in smokers in the SAI protocol. It can be assumed that both effects add up and are responsible for the observed excitability changes. Further studies should focus on different subgroups with different durations of abstinence or use ACh activating medication reducing withdrawal-related effects to discern the impact of abstinence from the effects of chronic nicotine consumption at least gradually.

A potentially confounding factor in both groups is antipsychotic medication. Some antipsychotic drugs are known to interfere in a direct or indirect way with neurotransmitter circuits underlying measures of

excitability (Foster et al., 2021). Both groups showed no significant differences in CPZ levels, however, sample sizes were limited and medication was heterogeneous.

One relevant factor could also be the large standard deviation in our measurements, which may have been caused by restlessness of the patients during the experiment. This was related to symptoms of withdrawal in smokers, medication side effects and illness-related restlessness in both groups. Controlling these effects in patients with schizophrenia could be addressed by larger samples. In addition, the number of MEP trials, which was in a medium range in our measurement, must be considered a limitation. More trials per protocol may reduce the standard deviation and further increase the quality of the data.

Furthermore, the SICI/ICF results must be seen with regard to the significant test pulse difference between the two groups. The standardization of the measurement on the test pulse should address these measurement differences, but accuracy effects on the results cannot be ruled out.

#### 4.2. Conclusion

Taken together, our results show changes in motor cortex excitability associated with chronic nicotine consumption in patients with schizophrenia and suggest altered cholinergic neurotransmission. These changes may partially explain increased smoking rates in schizophrenia as an attempt to compensate cholinergic deficits. Furthermore, this should be taken as a starting point for future research to test acute effects of nicotine on excitability patterns of patients, exploring also its therapeutic potential for non-smokers.

#### Conflict of interest

BP, SM, IP report no conflict of interest. MAN is member of the scientific advisory board of Neuroelectrics. FP is a member of the European Scientific Advisory Board of Brainsway Inc., and the International Scientific Advisory Board of Sooma. He has received speaker's honoraria from Mag&More GmbH, the neuroCare Group, and Brainsway Inc. His lab has received support with equipment from neuroConn GmbH, Ilmenau, Germany, Mag&More GmbH and Brainsway Inc. WS has received a speaker's honorarium from Mag&More GmbH and has been a member of an advisory board by Recordati Pharmaceutical Ltd. PF has received research support/honoraria for lectures or advisory activities from: Boehringer-Ingelheim, Janssen, Lundbeck, Otsuka, Recordati, Richter Pharma, Rovi, Sage und Takeda. AH has received paid speakerships from Recordati, Janssen, Otsuka and Lundbeck. He was member of Recordati, Otsuka, Lundbeck and Janssen advisory boards.

#### Contributions

AH, MAN and FP designed and directed the project, BP and SM carried out the experiments, performed the data analysis and took the lead in writing the manuscript, IP and WS contributed to the analysis of the results and to the writing of the manuscript, PF supervised the project. All authors provided critical feedback and helped shape the research, analysis and manuscript.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

## Acknowledgments

This study was funded by a grant of the Deutsche Forschungsgemeinschaft (DFG) to Alkomiet Hasan (HA 6091/5-1) and is part of the PhD (Dr. hum-biol) project of BP. MAN and FP were supported by the German Centre for Brain Stimulation (GCBS) research consortium [grant numbers 01EE1501, 01EE1403E and 01EE1403G], funded by the Federal Ministry of Education and Research (BMBF). MAN is supported by the Deutsche Forschungsgemeinschaft (DFG) (KU 3714/2-1).

## References

- Anstey, K.J., von Sanden, C., Salim, A., O'Keane, R., 2007. Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies. *Am. J. Epidemiol.* 166 (4), 367–378.
- Avram, M., Grothe, M.J., Meinhold, L., Leucht, C., Leucht, S., Borgwardt, S., Brandl, F., Sorg, C., 2021. Lower cholinergic basal forebrain volumes link with cognitive difficulties in schizophrenia. *Neuropsychopharmacology* 46 (13), 2320–2329.
- Brannan, S.K., Sawchak, S., Miller, A.C., Lieberman, J.A., Paul, S.M., Breier, A., 2021. Muscarinic Cholinergic Receptor Agonist and Peripheral Antagonist for Schizophrenia. *N. Engl. J. Med.* 384 (8), 717–726.
- Breese, C.R., Lee, M.J., Adams, C.E., Sullivan, B., Logel, J., Gillen, K.M., Marks, M.J., Collins, A.C., Leonard, S., 2000. Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. *Neuropsychopharmacology* 23 (4), 351–364.
- Bunse, T., Wobrock, T., Strube, W., Padberg, F., Palm, U., Falkai, P., Hasan, A., 2014. Motor Cortical Excitability Assessed by Transcranial Magnetic Stimulation in Psychiatric Disorders: A Systematic Review. *Brain Stimul.* 7 (2), 158–169.
- Bustillo, J.R., Rowland, L.M., Lauriello, J., Petropoulos, H., Hammond, R., Hart, B., Brooks, W.M., 2002. High choline concentrations in the caudate nucleus in antipsychotic-naïve patients with schizophrenia. *Am. J. Psychiatry* 159 (1), 130–133.
- Chapman, S., Ragg, M., McGeechan, K., 2009. Citation bias in reported smoking prevalence in people with schizophrenia. *Aust. N. Z. J. Psychiatry* 43 (3), 277–282.
- Coustals, N., Martelli, C., Brunet-Lecomte, M., Petillion, A., Romeo, B., Benyamina, A., 2020. Chronic smoking and cognition in patients with schizophrenia: A meta-analysis. *Schizophr. Res.* 222, 113–121.
- D'Souza, M.S., Markou, A., 2012. Schizophrenia and tobacco smoking comorbidity: nAChR agonists in the treatment of schizophrenia-associated cognitive deficits. *Neuropharmacology* 62 (3), 1564–1573.
- Dajas-Bailador, F., Wonnacott, S., 2004. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends Pharmacol. Sci.* 25 (6), 317–324.
- Dani, J.A., Bertrand, D., 2007. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* 47 (1), 699–729.
- de Leon, J., Diaz, F.J., 2005. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. *Schizophr. Res.* 76 (2–3), 135–157.
- Descarries, L., Gisiger, V., Steriade, M., 1997. Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* 53 (5), 603–625.
- di Hou, M., Santoro, V., Biondi, A., Shergill, S., Premoli, I., 2021. A systematic review of TMS and neurophysiological biometrics in patients with schizophrenia. *J. Psychiatry Neurosci.* JPN 46 (6), E675–E701.
- Dome, P., Lazary, J., Kalapos, M.P., Rihmer, Z., 2010. Smoking, nicotine and neuropsychiatric disorders. *Neurosci. Biobehav. Rev.* 34 (3), 295–342.
- Dondé, C., Brunelin, J., Mondino, M., Cellard, C., Rolland, B., Haesebaert, F., 2020. The effects of acute nicotine administration on cognitive and early sensory processes in schizophrenia: a systematic review. *Neurosci. Biobehav. Rev.* 118, 121–133.
- Durany, N., Zöchling, R., Boissl, K.W., Paulus, W., Ransmayr, G., Tatschner, T., Danielczyk, W., Jellinger, K., Deckert, J., Riederer, P., 2000. Human post-mortem striatal alpha4beta2 nicotinic acetylcholine receptor density in schizophrenia and Parkinson's syndrome. *Neurosci. Lett.* 287 (2), 109–112.
- Durazzo, T.C., Meyerhoff, D.J., Nixon, S.J., 2012. A comprehensive assessment of neurocognition in middle-aged chronic cigarette smokers. *Drug Alcohol Depend.* 122 (1–2), 105–111.
- Esterlis, I., Ranganathan, M., Bois, F., Pittman, B., Picciotto, M.R., Shearer, L., Anticevic, A., Carlson, J., Nicu, M.J., Cosgrove, K.P., D'Souza, D.C., 2014. In vivo evidence for  $\beta 2$  nicotinic acetylcholine receptor subunit upregulation in smokers as compared with nonsmokers with schizophrenia. *Biol. Psychiatry* 76 (6), 495–502.
- Even, N., Cardona, A., Soudant, M., Corringer, P.J., Changeux, J.P., Cloëz-Tayarani, I., 2008. Regional differential effects of chronic nicotine on brain alpha 4-containing and alpha 6-containing receptors. *Neuroreport* 19 (15), 1545–1550.
- Foster, D.J., Bryant, Z.K., Conn, P.J., 2021. Targeting muscarinic receptors to treat schizophrenia. *Behav. Brain Res.* 405, 113201.
- Freedman, R., 2014.  $\alpha 7$ -Nicotinic Acetylcholine Receptor Agonists for Cognitive Enhancement in Schizophrenia. *Annu. Rev. Med.* 65 (1), 245–261.
- García-Rill, E., Biedermann, J.A., Chambers, T., Skinner, R.D., Mrak, R.E., Husain, M., Karson, C.N., 1995. Mesopontine neurons in schizophrenia. *Neuroscience* 66 (2), 321–335.
- Grundy, J., Freznosa, S., Klinker, F., Lang, N., Paulus, W., Nitsche, M.A., 2013. Cortical excitability in smoking and not smoking individuals with and without nicotine. *Psychopharmacology* 229 (4), 653–664.
- Grundy, J., Barlay, J., Batsikadze, G., Kuo, M.-F., Paulus, W., Nitsche, M.A., 2018a. Nicotine modulates human brain plasticity via calcium-dependent mechanisms. *J. Physiol.* 596 (22), 5429–5441.
- Grundy, J., Thirugnasambandam, N., Amu, R., Paulus, W., Nitsche, M.A., 2018b. Nicotinic Restoration of Excitatory Neuroplasticity Is Linked to Improved Implicit Motor Learning Skills in Deprived Smokers. *Front. Neurol.* 9 (367).
- Guan, Z.Z., Zhang, X., Blennow, K., Nordberg, A., 1999. Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. *Neuroreport* 10 (8), 1779–1782.
- Hahn, B., Harvey, A.N., Concheiro-Guisan, M., Huestis, M.A., Holcomb, H.H., Gold, J.M., 2013. A test of the cognitive self-medication hypothesis of tobacco smoking in schizophrenia. *Biol. Psychiatry* 74 (6), 436–443.
- Hasan, A., Nitsche, M.A., Rein, B., Schneider-Axmann, T., Guse, B., Gruber, O., Falkai, P., Wobrock, T., 2011. Dysfunctional long-term potentiation-like plasticity in schizophrenia revealed by transcranial direct current stimulation. *Behav. Brain Res.* 224 (1), 15–22.
- Khedr, E.M., Tony, A.A., Abdelwarith, A., Safwat, M., 2020. Effect of chronic nicotine consumption on motor cortical excitability: A transcranial magnetic stimulation study. *Neurophysiol. Clin.* 50 (1), 33–39.
- Kujirai, T., Caramia, M.D., Rothwell, J.C., Day, B.L., Thompson, P.D., Ferbert, A., Wroe, S., Asselman, P., Marsden, C.D., 1993. Corticocortical inhibition in human motor cortex. *J. Physiol.* 471, 501–519.
- Lang, N., Hasan, A., Sueskes, E., Paulus, W., Nitsche, M.A., 2008. Cortical Hypoexcitability in Chronic Smokers? A Transcranial Magnetic Stimulation Study. *Neuropsychopharmacology* 33 (10), 2517–2523.
- Leucht, S., Samara, M., Heres, S., Davis, J.M., 2016. Dose Equivalents for Antipsychotic Drugs: The DDD Method. *Schizophr. Bull.* 42 Suppl 1(Suppl 1), S90–94. [https://academic.oup.com/schizophreniabulletin/article/42/suppl\\_1/S90/2413841?logi n=false](https://academic.oup.com/schizophreniabulletin/article/42/suppl_1/S90/2413841?logi n=false).
- Mathew, S.V., Law, A.J., Lipska, B.K., Dávila-García, M.I., Zamora, E.D., Mitkus, S.N., Vakkalanka, R., Straub, R.E., Weinberg, D.R., Kleinman, J.E., Hyde, T.M., 2007. Alpha7 nicotinic acetylcholine receptor mRNA expression and binding in postmortem human brain are associated with genetic variation in neuregulin 1. *Hum. Mol. Genet.* 16 (23), 2921–2932.
- McGehee, D.S., Heath, M.J., Gelber, S., Devay, P., Role, L.W., 1995. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* 269 (5231), 1692–1696.
- Noda, Y., Barr, M.S., Zomorodi, R., Cash, R.F.H., Rajji, T.K., Farzan, F., Chen, R., George, T.P., Daskalakis, Z.J., Blumberger, D.M., 2017. Reduced Short-Latency Afferent Inhibition in Prefrontal but not Motor Cortex and Its Association With Executive Function in Schizophrenia: A Combined TMS-EEG Study. *Schizophr. Bull.* 44 (1), 193–202. <https://academic.oup.com/schizophreniabulletin/article/44/1/193/3098109>.
- Oldfield, R.C., 1971. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 9 (1), 97–113.
- Oliveira, H., Coutinho, L., Higuchi, C., Noto, C., Bressan, R., Gadelha, A., 2018. T100. Nicotine use impacts negative symptoms severity in schizophrenia. *Schizophr. Bull.* 44(Suppl 1), S154. [https://academic.oup.com/schizophreniabulletin/article/44/suppl\\_1/S154/4957441](https://academic.oup.com/schizophreniabulletin/article/44/suppl_1/S154/4957441).
- Paul, R.H., Brickman, A.M., Cohen, R.A., Williams, L.M., Niaura, R., Pogun, S., Clark, C.R., Gunstad, J., Gordon, E., 2006. Cognitive status of young and older cigarette smokers: data from the international brain database. *J. Clin. Neurosci.* 13 (4), 457–465.
- Radhu, N., de Jesus, D.R., Ravindran, L.N., Zanjani, A., Fitzgerald, P.B., Daskalakis, Z.J., 2013. A meta-analysis of cortical inhibition and excitability using transcranial magnetic stimulation in psychiatric disorders. *Clin. Neurophysiol.* 124 (7), 1309–1320.
- Recio-Barbero, M., Segarra, R., Zabala, A., González-Fraile, E., González-Pinto, A., Ballesteros, J., 2021. Cognitive Enhancers in Schizophrenia: A Systematic Review and Meta-Analysis of Alpha-7 Nicotinic Acetylcholine Receptor Agonists for Cognitive Deficits and Negative Symptoms. *Front. Psych.* 12, 631589.
- Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P.B., George, M.S., Hallett, M., Lefaucheur, J.P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M.A., Pascual-Leone, A., Paulus, W., Rossi, S., Rothwell, J.C., Siebner, H.R., Ugawa, Y., Walsh, V., Ziemann, U., 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N Committee. *Clin. Neurophysiol.* 126 (6), 1071–1107.
- Sabia, S., Marmot, M., Dufouil, C., Singh-Manoux, A., 2008. Smoking history and cognitive function in middle age from the Whitehall II study. *Arch. Intern. Med.* 168 (11), 1165–1173.
- Sher, E., Chen, Y., Sharples, T.J., Broad, L.M., Benedetti, G., Zwart, R., McPhie, G.I., Pearson, K.H., Baldwin, T., De Filippi, G., 2004. Physiological roles of neuronal nicotinic receptor subtypes: new insights on the nicotinic modulation of neurotransmitter release, synaptic transmission and plasticity. *Curr. Top. Med. Chem.* 4 (3), 283–297.
- Shoyama, M., Takahashi, S., Hashimoto, T., Tsuji, T., Ukai, S., Shinosaki, K., 2013. Short latency afferent inhibition in schizophrenia patients. *Asean J. Psychiatry* 14 (2).
- Tani, M., Akashi, N., Hori, K., Konishi, K., Kitajima, Y., Tomioka, H., Inamoto, A., Hirata, A., Tomita, A., Koganemaru, T., Takahashi, A., Hachisu, M., 2015. Anticholinergic Activity and Schizophrenia. *Neurodegener. Dis.* 15 (3), 168–174.

- Terry, A.V., Callahan, P.M., 2020.  $\alpha 7$  nicotinic acetylcholine receptors as therapeutic targets in schizophrenia: Update on animal and clinical studies and strategies for the future. *Neuropharmacology* 170, 108053.
- Théberge, J., Al-Semaan, Y., Drost, D.J., Malla, A.K., Neufeld, R.W., Bartha, R., Manchanda, R., Menon, R., Densmore, M., Schaefer, B., Williamson, P.C., 2004. Duration of untreated psychosis vs. N-acetylaspartate and choline in first episode schizophrenia: a 1H magnetic resonance spectroscopy study at 4.0 Tesla. *Psychiatry Res.* 131 (2), 107–114.
- Turco, C.V., El-Sayes, J., Savoie, M.J., Fassett, H.J., Locke, M.B., Nelson, A.J., 2018. Short- and long-latency afferent inhibition; uses, mechanisms and influencing factors. *Brain Stimul.* 11 (1), 59–74.
- Turner, J.R., Castellano, L.M., Blendy, J.A., 2011. Parallel anxiolytic-like effects and upregulation of neuronal nicotinic acetylcholine receptors following chronic nicotine and varenicline. *Nicotine Tobacco Res.* 13 (1), 41–46.
- Wing, V.C., Wass, C.E., Bacher, I., Rabin, R.A., George, T.P., 2013. Varenicline modulates spatial working memory deficits in smokers with schizophrenia. *Schizophr. Res.* 149 (1), 190–191.
- Winterer, G., 2010. Why do patients with schizophrenia smoke? *Curr. Opin. Psychiatry* 23 (2), 112–119.
- Zarei, M.M., Radcliffe, K.A., Chen, D., Patrick, J.W., Dani, J.A., 1999. Distributions of nicotinic acetylcholine receptor  $\alpha 7$  and  $\beta 2$  subunits on cultured hippocampal neurons. *Neuroscience* 88 (3), 755–764.
- Zhang, X.Y., Chen, D.C., Xiu, M.H., Haile, C.N., Sun, H., Lu, L., Kosten, T.A., Kosten, T.R., Boraud, T., 2012. Cigarette smoking and cognitive function in Chinese male schizophrenia: a case-control study. *PLoS One* 7 (5), e36563.
- Ziemann, U., Reis, J., Schwenkreis, P., Rosanova, M., Strafella, A., Badawy, R., Müller-Dahlhaus, F., 2015. TMS and drugs revisited 2014. *Clin. Neurophysiol.* 126 (10), 1847–1868.