ORIGINAL ARTICLE

Does repetitive transcranial magnetic stimulation have a positive effect on working memory and neuronal activation in treatment of negative symptoms of schizophrenia?

Radovan Přikryl [1,2,5], Michal Mikl [1,3,6], Hana Přikrylová Kučerová [1,2,5], Libor Ustohal [1,2,5], Tomáš Kašpárek [1,2,5], Radek Mareček [1,3,6], Michaela Vrzalová [2,5], Eva Češková [1,2,5], Jiří Vaníček [4,6]

1 Ceitec-Masaryk University Brno, Czech Republic
2 Department of Psychiatry, Faculty of Medicine Masaryk University Brno, Czech Republic
3 Department of Neurology, Faculty of Medicine Masaryk University Brno, Czech Republic
4 Department of Imaging Methods, Faculty of Medicine Masaryk University Brno, Czech Republic
5 University Hospital Brno, Czech Republic
6 St. Anne´s University Hospital, Brno, Czech Republic

Correspondence to: Radovan Prikryl
Department of Psychiatry University Hospital Brno
Jihlavská 20, 625 00 Brno, Czech Republic.
tel: +420 5 3223 2055; fax: +420 5 3223 3706; e-mail: radovan.prikryl@post.cz

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Abstract

OBJECTIVE: The objective of the study was to find out whether, under the conditions of a double-blind, placebo coil controlled study, high-frequency repetitive transcranial magnetic stimulation (TMS) over the left prefrontal cortex will show positive effects on working memory with simultaneous assessment of respective changes in neuronal activation.

RESULTS: Stimulation treatment led to a reduction of seriousness of negative schizophrenia symptoms in both comparative groups. However, mutual comparison of real (n=19) and sham (n=11) rTMS, respectively, has shown that the effect of real rTMS was statistically significantly higher compared with placebo stimulation. During stimulation treatment an improvement in working memory performance was also found. No statistically significant difference between the real and placebo sham rTMS, respectively, was established. The rate of neuronal activation did not change at all during rTMS treatment.

CONCLUSIONS: From clinical point of view rTMS seems to be a well-tolerated neurostimulation method for treatment of negative schizophrenia symptoms with favourable of impact on cognitive functions.

INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) represents a promising therapeutic method for influencing negative symptoms of schizophrenia, thanks to its unique ability to modulate the neuronal activity of the cortical cerebral areas and neuronal spheres that are included in the pathophysiology of schizophrenia, both directly and indirectly by means of trans-synaptic transfer (Prikryl *et al.* 2011a;b). During treatment of negative schizophrenia symptoms the high-frequency (10 Hz) rTMS over the left dorsolateral prefrontal cortex (DLPFC) area has been proven to be particularly useful (Dlabac *et al.* 2010). The hypofunction of the left DLPFC is simultaneously considered a neuronal correlate of several cognitive deficit domains in schizophrenia, e.g. working memory, verbal fluency and executive functions (Lui *et al.* 2010; Dusek *et al.* 2011). While it is known that high frequency rTMS over the left DLPFC area is able to modify hypofrontality, its effect on cognitive functions remains ambiguous (Guse *et al.* 2010).

Therefore, during treatment of negative schizophrenia symptoms by means of high-frequency rTMS over the left DLPFC area, a question arises if, in addition to the influence on negative symptoms, rTMS also shows an effect on certain parameters of cognitive schizophrenia deficit. The theoretical justification of such efficacy may be particularly seen in the fact that the negative symptoms and cognitive deficit share a series of common characteristics. The relevance of negative symptoms correlates positively with cognitive deficit rate in patients with schizophrenia. Negative symptoms are considered by some authors to be a result of the basic cognitive deficit, manifested on the level of behavior (Carpenter *et al.* 1988; Andreasen *et al.* 1997). In addition, it seems that they may have a common or at least similar pathogenesis. Actually, most of the functional neuroimaging studies ascribe the etiopathogenesis of negative symptoms and cognitive schizophrenia deficit, especially working memory, executive functions or verbal fluency, to a disturbed left DLPFC function (Callicott *et al.* 2000), and possibly that of the medial prefrontal cortex (Delamilliere *et al.* 2000). Also, a deficit of dopaminergic neurotransmission in the prefrontal cortex, which results in disruption of signal transfer due to dysfunctional, up-regulated dopamine D_1 receptors, is considered a probable etiopathogenetic base of negative symptoms and cognitive schizophrenia symptoms (Weinberger *et al.* 1988; Abi-Dargham 2002).

It is therefore relatively surprising that there is very little experience with the rTMS effect on cognitive functions compared with negative schizophrenia symptoms treatment. With the exception of the work of Mittrach, who is the only one who primarily focused on cognitive functions within the scope of rTMS tolerance assessment during schizophrenia treatment (Mittrach *et al.* 2010), it is only possible to arise from partial results of five studies whose principal objective was, however, to verify rTMS efficacy in negative schizophrenia treatment and in which cognitive functions were assessed only within the scope of secondary tasks of those studies (Cohen *et al.* 1999; Rollnik *et al.* 2000; Holi *et al.* 2004; Sachdev *et al.* 2005; Novak *et al.* 2006). In addition, the principal objective of the research did not involve procognitive rTMS effects but rather in an answer to a question whether rTMS disrupts cognitive functions. The only study dealing with the direct impact of rTMS on cognitive schizophrenia deficit was a study of Mohr who, despite previous studies, did not primarily concentrate on the impact on schizophrenia symptoms but only compared possible procognitive effects of rTMS compared with efficacy of computer rehabilitation of cognitive schizophrenia deficit (Mohr *et al.* 2006). Studies aimed at the impact of high-frequency rTMS, administered over the left DLPFC, on cognitive schizophrenia deficits did not show a positive effect on cognitive functions in most cases (Holi *et al.* 2004; Sachdev *et al.* 2005; Mohr *et al.* 2006; Novak *et al.* 2006). An open, pilot study found improvement of cognitive functions during stimulation treatment, however, statistical significance was only reached by delayed visual reproduction (Cohen *et al.* 1999). On the other hand, a single blind, placebo "sham" controlled study found only a trend toward reduction of cognitive deficit in cases of real stimulation, but without any statistical difference between real and inefficient sham stimulation (Rollnik *et al.* 2000). A similar conclusion was also found by a double blind study assessing primarily the effect of high frequency rTMS over the left DLPFC area on cognitive functions. It did not detect any statistically significant deterioration of cognitive performance during rTMS treatment, on the contrary, in the patients treated with real rTMS a trend toward improvement in performance in executive functions, correlating with psychopathology, was found (Mittrach *et al.* 2010).

During treatment of negative schizophrenia symptoms by means of high frequency rTMS over the left DLPFC area, positive effects of stimulation on disturbed prefrontal cortex metabolism is considered to be one of the possible mechanisms of action. However, the performed studies have not found any such evidence. Actually, a combination of functional neuroimaging methods with rTMS treatment of negative symptoms has only been used in two studies. The first of them was a small open study with six patients in whom cerebral perfusion tested with the activation cognitive paradigm using the Wisconsin card sorting test (WCST) and one-photon emission computer tomography (SPECT) was measured. Even if a statistically significant decrease in seriousness of negative symptoms occurred, the clinical effect of such a change was assessed as small and hypofrontality, demonstrated by means of SPECT, did not change at all during treatment (Cohen *et al.* 1999). The other work was a double blind study with a sham coil, in which cerebral perfusion was also measured by means of SPECT in order to be able to establish whether rTMS

influences regional blood flow through the brain. In patients treated with real rTMS more sizable decrease in negative schizophrenia symptoms occurred in comparison with placebo stimulation, however, the SPECT data analysis did not reveal any significant changes in the regional cerebral perfusion connected with real or placebo stimulation (Hajak *et al.* 2004).

As far as the authors know, no double blind study aimed at evaluation of high frequency rTMS over the left DLPFC on cognitive schizophrenia deficit in combination with functional magnetic resonance imaging (fMRI) has been carried out. The principal objective of the study was to find out whether, under conditions of a double-blind, placebo coil controlled study, high frequency rTMS over the left DLPFC would have a positive effect on working memory, as assessed by means of the Verbal Fluency Test (VFT), an important parameter of cognitive schizophrenia deficit with simultaneous examination of the respective changes of neuronal activation by means of fMRI.

METHODS

Group parameters

The evaluated group included 30 patients (males) who were admitted to hospital, due to schizophrenia, at the Psychiatric clinic of the Faculty of Medicine of Masaryk University and Faculty Hospital in Brno, Czech Republic. Only those patients who fulfilled the criteria for schizophrenia (F20) according to the International Classification of Diseases, revision 10 (ICD-10) and diagnostic research criteria and who were stabilized long-term (for at least 6 weeks) on antipsychotics, with significant negative symptoms without other psychiatric comorbidity such as mood, anxiety or personality disorders and had no contraindications to magnetic resonance examination were included in the study. The diagnosis was verified by two independent experienced psychiatrists. The age of the enrolled patients ranged from 18 to 60 years. Patients who had a neurological disease (including epilepsy or an abnormal EEG record), cardiovascular, cerebrovascular, endocrinal, systemic autoimmune disease, psychoactive drug abuse including alcohol or acute risk of suicide at screening or in the past were not included in the study. Absence of psychoactive drug abuse was verified using a toxicology examination of urine for cannabis, amphetamines and opioids. Only those patients who signed an informed consent document and who had no contraindication for rTMS were included in the study. The study was approved by the local ethics committee and complies with the requirements of the Declaration of Helsinki.

Study procedures and treatment design

The evaluated group was divided in two subgroups using a random number generator. The first subgroup (19 patients) received real rTMS and the second subgroup (11 patients) received sham rTMS. Both forms of rTMS treatment were performed on workdays, i.e. 5 times a week, until completion of 15 procedures. During the stimulation therapy, patients remained on their prescribed antipsychotic medication. The working memory performance was assessed by an experienced neuropsychologist before and after rTMS treatment using the VFT (letters N, K, P) (Prescott *et al.* 2006). The severity of psychopathological symptoms before and after treatment was evaluated using the PANSS scale (Positive and Negative Syndrome Scale) (Kay *et al.* 1987). At the same times (before and after rTMS treatment) patients underwent a fMRI investigation. The evaluation of the psychopathology, working memory, fMRI data processing and performance of rTMS were mutually blinded.

Method of performance of rTMS

The rTMS treatment commenced with determination of the patient's motor threshold (MT) and localization of the site of stimulation. Motor threshold is determined using the electromyograph (EMG) in the musculus abductor pollicis brevis l. dx. Determination of MP is defined as the lowest stimulation activity that causes at least 5 motor potentials with an amplitude at least 50 mV in 10 single impulses. The actual performance of rTMS is defined by the location of application (left dorsolateral prefrontal cortex), intensity of magnetic stimulation in % of MP (110% of MT), stimulation frequency (10 Hz), duration of impulse series (10 s), interval between sequences (30 s) and the total number of applied stimuli (1 500). The clinical status and technical data of the application including temperature of the stimulation coil were observed over the whole application. The Magstim Super Rapid stimulation device was used for rTMS, and EMG MedelecSynergy was used for evaluation of the motor threshold. Sham stimulation was carried out in a similar manner by using a sham coil system without induction of a magnetic field. The sham procedure elicited no tactile sensation at the site of stimulation and guaranteed that no substantial cortical stimulation occurred.

Method of fMRI investigation: VFT activation task

A silent phonemic verbal fluency task (VFT) was used as a language paradigm. We used a block design, with active tasks alternating with periods of rest. Active as well as control blocks lasted 32 seconds. Five blocks of each condition were performed. In the active block, subjects silently generated as many words as possible, beginning with one of the following letters, O, K, P, N, I, that had been presented to them visually through a data projector during the whole active period. During the rest periods (indicated by three asterisks '***') subjects simply relaxed. After scanning, subjects were asked whether they had performed the task successfully. All subjects reported that they generated words for all presented letters.

Method of fMRI investigation: acquisition parameters

Imaging was performed on a 1.5 T MR scanner (Siemens Symphony – Erlangen, Germany) equipped with a Numaris 4 System (MRease). Each subject underwent the same fMRI examination twice – before and after rTMS. The functional images were acquired using a gradient echo, echoplannar (EPI) sequence with the following parameters: TR (scan repeat time) = 4 520 ms, TE=40 ms, FOV=220 mm, flip angle 90°, matrix size 64 × 64, slice thickness 3.5 mm, 32 transversal slices per scan, 71 scans per each functional measurement (7 scans per each block and one dummy scan at the beginning of acquisition). High-resolution anatomical T1-weighted images, which served as a matrix for the functional imaging, were acquired using a 3D sequence with the parameters: TR=1 700 ms, TE=3.96 ms, FOV=246 mm, flip angle 15°, resolution 256 × 256 resampled to 512 × 512, slice thickness 1.17 mm, 160 sagittal slices. The subjects were instructed to remain still while in the scanner.

Method of fMRI data statistical analysis

The data was analyzed using SPM5 software (Functional Imaging Laboratory, the Wellcome Department of Imaging Neuroscience, Institute of Neurology at University College London, UK) running under Matlab 7.6 (Mathworks Inc., USA). The following pre-processing was applied to each subject's time series of fMRI scans: realignment and unwarping of functional scans to correct for head movement; normalization to fit into a standard anatomical space (MNI), and spatial smoothing using a Gaussian filter with a FWHM of 8 mm. The voxel size generated from the above acquisition parameters was resampled to 3 × 3 × 3 mm. To determine the brain regions that showed significant activation with respect to active periods, a General Linear Model (GLM) as implemented in the SPM5 was used. The experimental stimulation time-course was convolved with a canonical hemodynamic response function. A high-pass filter with a cut-off at 128 s and an autoregressive model to estimate serial correlations were used within the GLM. The movement parameters obtained during realignment were used as nuisance variables (regressors of no interest) in the design matrix. Statistical parametric maps with t-statistics were computed to assess the effects of activation (BOLD signal increase) with respect to the active condition. Corresponding contrast files were carried out in the second-level analyses. We used a random-effect analysis as implemented in the SPM5 to assess the effect of real rTMS. This was performed using a flexible factorial design (ANOVA) with three factors – subject, group, and condition. The group factor indicated the presence to group with real or sham rTMS. The condition factor indicated whether the functional measurement was performed before or after rTMS. These factors, interaction group x condition, and the covariate (behavioral VFT) were used in the design matrix. We were interested in the effect of interaction. The results were assessed using cluster-level inference at the $p<0.05$ level of significance, FWE corrected.

RESULTS

Characteristics of the evaluated group

The evaluated group included in total 30 patients (males). Toxicology examination of urine for cannabis, amphetamines and opioids was negative in all patients. All patients fulfilled the inclusion criteria for the study and no contraindications for rTMS and fMRI were found. All participants signed an informed consent document for the study.

Demographic data of the real rTMS group

The real rTMS group included 19 patients (mean age 30.47 years; SD=9.19) with education defined by the number of years of compulsory education (mean 10.63 years; SD=2.52) and mean duration of disease (4.05 years; SD=1.51). The mean daily dose of antipsychotics was 328.95 mg (SD=143.44) of chlorpromazine equivalents (see Table 1).

Demographic data of the sham rTMS group

The sham rTMS group included 11 patients (mean age 34.55 years; SD=10.57) with education defined by the number of years of compulsory education (mean 11.09 years; SD=1.51) and mean duration of disease (4.18 years; SD=1.89). The mean daily dose of antipsychotics was 304.55 mg (SD=140.01) of chlorpromazine equivalents (see Table 1).

Comparison of demographic data of real and sham rTMS groups

No statistically significant differences were found between the demographic data of the two groups of patients (see Table 1).

Characteristics of the working memory performance and the clinical status of the real rTMS group

Table 2 shows the mean scores on the VFT and PANSS before and after rTMS in the real rTMS group. A statistically significant increase in the mean VFT score and statistically significant reduction of severity of the negative, general and total symptoms of schizophrenia occurred in the real rTMS group. The rate of positive symptoms of schizophrenia remained unchanged (see Table 2).

Characteristics of the working memory and the clinical status of the sham rTMS group

Table 3 shows the mean scores of the VFT and PANSS before and after rTMS in the sham rTMS group. A statistically significant increase in the mean VFT score and statistically significant reduction of severity of general and total symptoms of schizophrenia occurred in the sham rTMS group. The intensity of the negative symptoms of schizophrenia was not statistically significantly

reduced. The rate of positive symptoms of schizophrenia remained unchanged (see Table 3).

Comparison of the size of ongoing changes of the working memory and the clinical status between the real and sham rTMS groups

Compared to sham rTMS, real rTMS caused a statistically significantly higher reduction in the severity of negative and total symptoms of schizophrenia. On the other hand, no difference was found with respect to the VFT, positive and common symptoms of schizophrenia (see Table 4).

Comparison of the size of ongoing changes of the neuronal activations between the real and sham rTMS groups

Figure 1 shows patterns of neuronal activations before and after stimulation treatment in the real and sham rTMS group. When VFT was burdened by the cognitive paradigm, the neuronal areas of the frontal cingulum were activated (Brodmann area 24) and the left DLPFC cortex (Brodmann area 9 and 46). No statistically significant differences in the neuronal activation size during rTMS treatment neither in real nor in sham rTMS groups were found.

DISCUSSION

The objective of the study was to establish whether high frequency rTMS, applied over the left DLPFC area, shows, in addition to reduction in seriousness of negative schizophrenia symptoms, a potential to positively modulate working memory capacity. Its advantage lies in the fact that it does not concentrate on behavioral data only, but also on possible changes in the neuronal activation detected by means of fMRI using VFT as a cognitive paradigm.

Tab. 1. Comparison of demographic data of the groups treated with real and sham rTMS.

	Real rTMS		Sham rTMS		Statistical comparison*	
	Mean	S.D.	Mean	S.D.	Z	*p*-value
Age (years)	30.47	9.19	34.55	10.57	0.97	0.33
Education (number of years)	10.63	2.52	11.09	1.51	1.21	0.23
Duration of schizophrenia (years)	4.05	1.51	4.18	1.89	0.09	0.93
Daily dose of antipsychotics (in mg CHLO EQ.)	328.95	143.44	304.55	140.01	0.37	0.72

* Mann-Whitney U Test

Tab. 2. Mean scores of the VFT and PANSS before and after rTMS in the real rTMS group.

Scale	Before rTMS		After rTMS		Statistical comparison*	
	Mean	S.D.	Mean	S.D.	Z	*p*-value
VFT	37.95	14.54	44.68	14.78	2.96	0.01
Positive subscale PANSS	8.16	1.80	7.79	1.27	1.35	0.18
Negative subscale PANSS	23.16	5.11	16.26	5.79	3.72	0.01
General subscale PANSS	33.05	6.09	26.84	5.69	3.29	0.01
Total PANSS	64.37	9.70	50.89	14.78	3.70	0.01

* Wilcoxon Matched Pairs Test: comparison of the scores before and after rTMS
VFT (Verbal Fluency Task), PANSS (Positive and Negative Syndrome Scale)

Tab. 3. Mean scores of the VFT and PANSS before and after rTMS in the sham rTMS group.

Scale	Before rTMS		After rTMS		Statistical comparison*	
	Mean	S.D.	Mean	S.D.	Z	*p*-value
VFT	37.82	9.68	43.82	11.39	2.27	0.02
Positive subscale PANSS	8.09	1.92	8.27	2.28	0.27	0.79
Negative subscale PANSS	22.09	4.18	21.36	4.08	0.94	0.34
General subscale PANSS	33.27	4.65	30.00	4.31	2.60	0.01
Total PANSS	63.45	9.22	59.63	8.10	1.99	0.05

* Wilcoxon Matched Pairs Test: comparison of the scores before and after rTMS
VFT (Verbal Fluency Task), PANSS (Positive and Negative Syndrome Scale)

Tab. 4. Comparison of changes of the mean scores (in percentage reduction) of the VFT and PANSS between the groups treated with real and sham rTMS.

	Real rTMS		Sham rTMS		Statistical comparison*	
	Mean (%)	S.D.	Mean (%)	S.D.	Z	*p*-value
VFT	21.80%	24.66	16.89%	19.44	0.26	0.80
Positive subscale PANSS	−3.09%	11.54	2.58%	16.01	−0.90	0.36
Negative subscale PANSS	−30.25%	17.41	−2.66%	11.20	−3.83	0.01
General subscale PANSS	−17.49%	16.48	−9.47%	9.15	−1.68	0.09
Total PANSS	−20.32%	12.91	−5.56%	8.86	−3.06	0.01

* Mann-Whitney U Test
VFT (Verbal Fluency Task), PANSS (Positive and Negative Syndrome Scale)

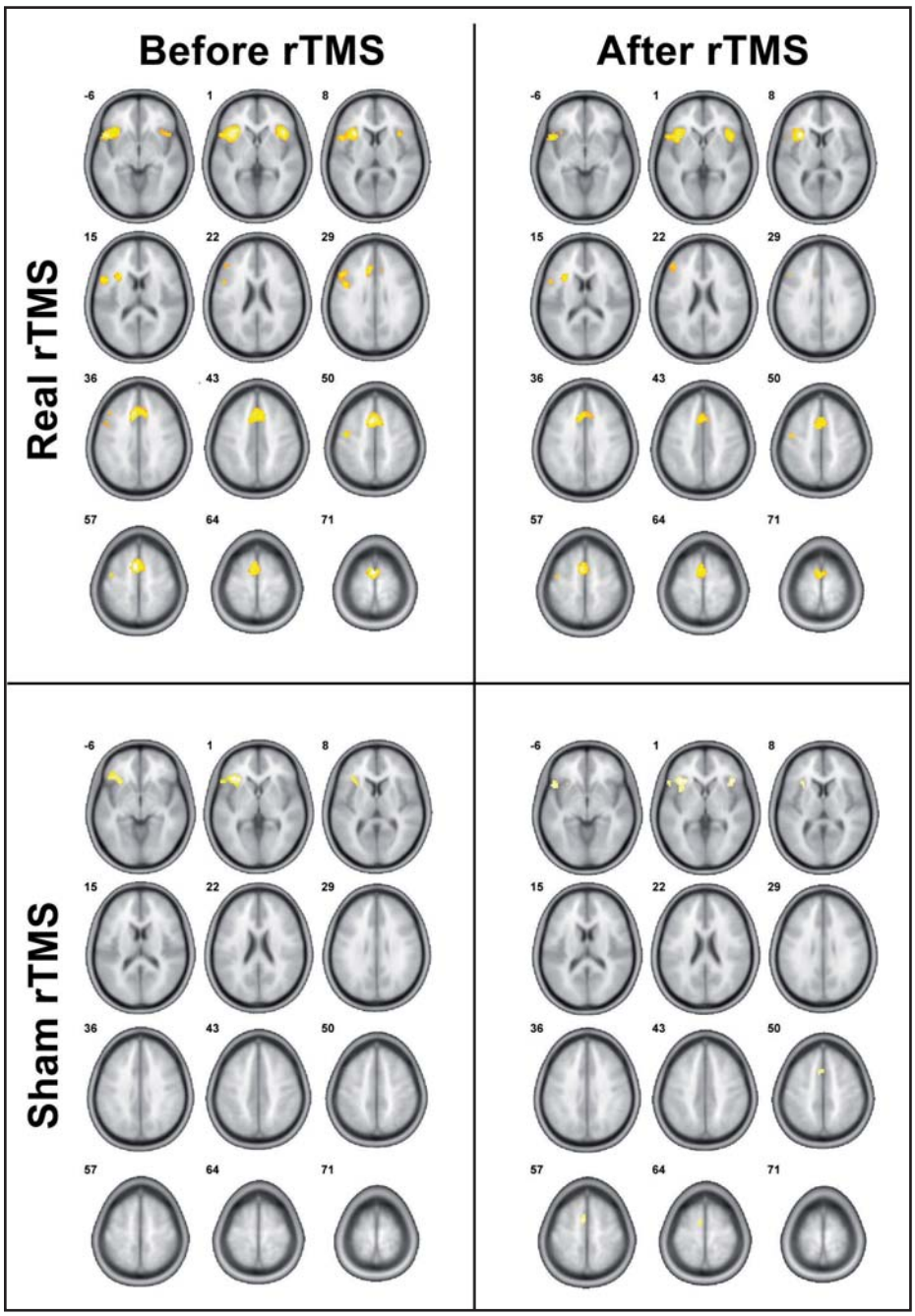

Fig. 1. Patterns of neuronal activations before and after stimulation treatment in the real and sham rTMS group.

Stimulation treatment led to a decrease in negative schizophrenia symptoms in both groups. However, comparison of real and sham rTMS revealed that the effect of real rTMS was statically more significant in comparison to the placebo one. This result indicates a placebo effect which accompanies rTMS in treatment of negative symptoms. Therefore the results of open studies dealing with impact on negative schizophrenia symptoms should be assessed having this fact in mind. Our finding also fully corresponds with conclusions of published meta-analyses which, based on placebo sham stimulation controlled studies carried out so far, demonstrated a moderate effect of rTMS in treatment of negative schizophrenia symptoms (Freitas *et al.* 2009; Dlabac *et al.* 2010).

Also, from the point of view of cognitive functions in both groups, an improvement of working memory performance, expressed by a statistically significant increase in the average VFT score, occurred during stimulation treatment. However, no statistically important difference between real and placebo sham rTMS was demonstrated during mutual comparison. The results indicate that the improvement of working memory in both real and placebo sham rTMS may be ascribed to the internship effect, which has been repetitively described for the VFT test (Cunje *et al.* 2007). That is, mutual comparisons have shown that high frequency rTMS over the left DLPFC area does not have any potential to significantly improve working memory performance. This finding also corresponds with the conclusions of other published studies which have not demonstrated a clear procognitive effect in schizophrenia (Holi *et al.* 2004; Sachdev *et al.* 2005; Mohr *et al.* 2006; Novak *et al.* 2006). Despite the previous works, we focused exclusively on working memory because its neuronal correlate was exactly the left DLPFC, which was the primary target of stimulation treatment.

The studies on healthy volunteers indicate an interesting finding. Actually, the action of high frequency rTMS over the left DLPFC led to a disruption of cognitive performance in most studies. Exceptions are found only in studies including young volunteers (Evers *et al.* 2001; Huang *et al.* 2005). It is interesting that the age factor also plays an important role in the intensity of the anti-depressive effect of rTMS (Figiel *et al.* 1998; Kozel *et al.* 2000; Janicak *et al.* 2002). The generally higher effect of rTMS is probably induced by higher flexibility and adaptability of the neuronal network in young individuals. Since only young patients were included in our population (the average age of the patients treated with real rTMS was 30 and that of the patients treated with placebo sham rTMS was 34), it can be expected that this fact contributed to the positive impact of rTMS on working memory.

Conclusions from studies dealing with rTMS impact on cognitive functions in treatment of the depressive disorder have mainly observed that high frequency rTMS, administered over the left DLPFC area, does not lead to any disruption of cognitive functions. On the contrary, a series of studies have shown improvement of cognitive deficits during stimulation treatment. The most considerable effect was reached mainly in parameters of memory/executive functions, which means in cognitive domains whose important neurobiological correlate is exactly dysfunction of the prefrontal cortex. The rate of change of cognitive functions in most studies has been connected with the extent of the antidepressive effects of rTMS (Little *et al.* 2000; O'Connor *et al.* 2005), but this is not a rule (Speer *et al.* 2001). It may be rather expected that the improvement of cognitive deficit in depressive disorder is more linked to remission of depressive symptomatology than to possible specific procognitive effects of rTMS.

Unlike the cognitive deficit, it seems that in cases of depression, rTMS does not have sufficient potential to influence cognitive schizophrenia deficits. An explanation is probably seen in the different causes of cognitive deficits in depressive disorder and in schizophrenia. While in case of depressive disorder, cognitive deficit is rather secondary and is, to a large extent, conditioned by the basic symptoms of depressive syndrome, which is particularly supported by its marked improvement after depression subsides (Kucerová *et al.* 2006). In schizophrenia cognitive deficit is a basic, core manifestation of the disease which does not subside with subsidence of other schizophrenia symptoms (Green *et al.* 1999; Keefe *et al.* 2000; Gold *et al.* 2004).

Our findings in the area of functional neuroimaging are also in accordance with those conclusions, which have been mainly drawn on the basis of neuropsychological testing and respective clinical data. Even when, the VFT was burdened by the cognitive paradigm, the expected cerebral areas were activated (frontal cingulum and the left DLPFC), neither real nor placebo sham rTMS treatment led to significant changes of neuronal activation. This finding supports our behavioral results in the sense that the changes in working memory performance are only caused by the internship effect and that rTMS probably does not have real procognitive potential for working memory parameters. Despite the fact that it was not the area of our research, it may be still expected that rTMS does not show the procognitive effect on other parameters of cognitive schizophrenia deficit either (Mittrach *et al.* 2010).

CONCLUSION

It can be concluded that we have not demonstrated any positive impact of high frequency rTMS, administered over the left DLPFC area, on working memory parameters and the respective changes of neuronal activation detected by means of fMRI. However, we have confirmed conclusions of previous works that considered our selected stimulation parameters of rTMS effective for treatment of negative schizophrenia symptoms (Freitas *et al.* 2009; Dlabac *et al.* 2010). From the clinical position, rTMS seems to be a very well tolerated

neurostimulation method for treatment of negative schizophrenia symptoms from the point of view of the effect on cognitive functions.

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