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Effect of Bilateral Prefrontal rTMS on Left Prefrontal NAA and Glx Levels in Schizophrenia Patients with Predominant Negative Symptoms: An Exploratory Study

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ABSTRACT

Background: Prefrontal repetitive Transcranial Magnetic Stimulation (rTMS) may improve negative symptoms in patients with schizophrenia, but few studies have investigated the underlying neural mechanism.
Objective: This study aims to investigate changes in the levels of glutamate and glutamine (Glx, neurotransmitter and precursor) and N-Acetyl Aspartate (NAA) in the left dorsolateral prefrontal cortex of patients with schizophrenia treated with active bilateral prefrontal rTMS as compared to sham-rTMS, as measured with ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS).
Methods: Patients were randomized to a 3-week course of active or sham high-frequency rTMS. Pre-treatment and post-treatment ^{1}H-MRS data were available for 24 patients with schizophrenia with moderate to severe negative symptoms (Positive and Negative Syndrome Scale (PANSS) negative subscale ≥ 15). Absolute metabolite concentrations were calculated using LCModel with the water peak as reference. To explore the association between treatment condition and changes in concentration of Glx and NAA, we applied a linear regression model.
Results: We observed an increase of Glx concentration in the active treatment group and a decrease of Glx concentration in the group receiving sham treatment. The association between changes in Glx concentration and treatment condition was significant. No significant associations between changes in NAA and treatment condition were found.
Conclusions: Noninvasive neurostimulation with high-frequency bilateral prefrontal rTMS may influence Glx concentration in the prefrontal cortex of patients with schizophrenia. Larger studies are needed to confirm these findings and further elucidate the underlying neural working mechanism of rTMS.

Introduction

Negative symptoms of schizophrenia are difficult to treat and are associated with poor functional outcome [1,2]. Several studies found that negative symptoms of schizophrenia are associated with dysfunction of the prefrontal cortex [3,4], which may be reflected by an imbalance of the neuronal metabolism of the prefrontal cortex. Indeed, neuroimaging studies have found a relationship between a reduced N-Acetyl Aspartate (NAA) concentration in the frontal cortex and severity of negative symptoms [5–7]. Glutamatergic dysfunction has also been suggested to be associated with negative symptoms [8].

Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive technique to modulate neuronal activity. rTMS involves the use of alternating magnetic fields at a set frequency in order to induce an electric current in the underlying brain tissue. Several applications with rTMS for the treatment of psychiatric disorders have been investigated in recent years [9], among which the treatment of neg-

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ative symptoms of schizophrenia with high frequency prefrontal rTMS. Four meta-analyses have been conducted on published trials of rTMS for negative symptoms. One, based on five studies, did not find a significant improvement after rTMS treatment as compared to sham treatment [10]. A second meta-analysis, which included seven studies, found a trend toward improvement of negative symptoms [11]. The other two meta-analyses, including nine and thirteen studies respectively, found a positive effect of prefrontal high frequency rTMS for the treatment of negative symptoms of schizophrenia [12,13]. Recently, two large randomized controlled trials on rTMS treatment of negative symptoms have been conducted [14,15]. One trial did not find any improvement of negative symptoms [15] or cognition [16] after a 3-week treatment with 10 Hz rTMS. The other trial did find an improvement of negative symptoms after four weeks of 10 Hz, 20 Hz and theta burst stimulation as compared to sham stimulation [14]. In the latter study by Zhao et al. at least twice as many stimuli were applied than the study by Wobrock et al. [15] and treatment duration was one week longer. In addition, the PANSS negative symptom score of their study was very high (mean score above 36) compared to previous studies, including the study by Wobruck et al. (mean score of 25). Thus, although results are inconsistent, there is a reasonable amount of evidence that rTMS may be an effective treatment option for negative symptoms of schizophrenia. Considering the above, it seems important to further investigate optimal rTMS treatment parameters and its mechanism of action, which includes effects on brain metabolism.

^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS) can measure metabolite concentrations in the brain. Prefrontal rTMS has been found to increase cortical glutamate and glutamine levels in healthy volunteers [17] and in patients with a depression [18]. Interestingly, in patients suffering from depression it was observed that these changes were dependent on pre-treatment glutamate and glutamine concentrations. An increase of NAA in the anterior cingulate has also been reported in treatment-resistant major depressive disorder after rTMS [19].

The objective of this exploratory study was to investigate changes in the levels of glutamate and glutamine (Glx, neurotransmitter and precursor) and NAA in the left dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia and negative symptoms treated with active or sham bilateral rTMS.

Materials and methods

Participants

The current MRS study was part of a larger double-blind randomized controlled trial (Dutch Trial Register NTR 1261) on rTMS treatment of negative symptoms conducted among 32 patients, which found a positive treatment effect up to three months follow-up [20]. From a subsample of 24 patients, pre-treatment and post-treatment ^{1}H-MRS data were available. The MRS data of the remaining patients could not be included in the analyses for several reasons, including unavailability of the scanner due to an update (N = 1), no-show (N = 2), claustrophobia (N = 1), low spectral quality (N = 1) and technical problems during scanning (N = 3).

Patients were recruited for this trial from in- and outpatient facilities of three regional mental health care institutions (Lentis, GGz Drenthe and GGz Friesland) and the University Medical Center Groningen (UMCG). Patients were 18 years or older, and all met the DSM-IV criteria for schizophrenia or schizoaffective disorder confirmed by a trained interviewer using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) [21]. Furthermore, patients were included if their negative sub-score was equal or larger than 15 on the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) [22]. Exclusion criteria were rTMS and MRI contraindications, neurological disorders, head injury with loss of consciousness in the past, substance dependency within the previous 6 months, previous treatment with rTMS, severe behavioral disorders, inability to provide informed consent and pregnancy. Patients were stable on medication for at least 6 weeks prior to participating in the study and for the duration of the study. The study was executed in accordance with the Declaration of Helsinki and approved by the local medical ethical committee of the University Medical Center of Groningen (UMCG). Participants provided oral and written consent after the procedure had been fully explained and was understood.

Study design

Participants were randomized to receive either active or sham rTMS treatment. Sequentially numbered sealed envelopes, which contained tokens drawn by an independent colleague, were used to conceal allocation. Raters and patients were kept blind to treatment condition, only the researcher and the trained nurses administering the rTMS were aware of the treatment condition. After treatment, all participants were asked to fill in a questionnaire about which treatment (sham or active treatment) they thought they had received to check for blinding success.

rTMS protocol

rTMS was administered using a Medtronic MagPro X100 stimulator with a 75 mm figure-eight coil. Patients were stimulated at a frequency of 10 Hz in 10-s trains, with an inter-train interval of 50 s. Patients were stimulated 20 min per session, during the morning session the left DLPFC was stimulated and during the afternoon session the right DLPFC was stimulated. Each participant underwent 30 treatment sessions, two sessions a day, for three consecutive weeks (workdays only), resulting in the administration of a total of 60.000 pulses. Stimulation intensity was set at 90% of the motor threshold [23]. The F3 and F4 location from the EEG 10–20 system were used to target the bilateral DLPFC [24]. For sham stimulation, the coil was tilted 90° off the scalp with two wings of the coil touching the scalp.

Negative symptoms

Negative symptoms were measured with the Scale for the Assessment of Negative Symptoms (SANS) [25] and the PANSS negative symptoms subscale. Ratings were performed at baseline, post-treatment, at four weeks follow-up and at three months follow-up.

Image acquisition

The first MRS measurement was performed in the week before the start of the treatment, the second one day after the last rTMS treatment session. The MRI scans were acquired at the NeuroImaging Center of the UMCG in Groningen using a 3T Philips Intera MRI scanner (Best, the Netherlands), equipped with a synergy SENSE eight-channel head coil. ^{1}H-MRS single-voxel spectroscopy was acquired with an 8 cm^{3} voxel in order to assess proton metabolites in the white matter of the left DLPFC. The voxel was placed in line with the genu of the corpus callosum on the anterior side and oriented in the same line as the corpus callosum and the falx cerebri, see Fig. 1. Inclusion of white matter was maximized. A Point Resolved Spectroscopy (PRESS) sequence was used, with one 90° and two 180° pulses to create a spin echo, and water suppression with an excitation pulse followed by a frequency-modulated pulse. Automated standardization of the field in the examined region of interest (pencil beam auto first order option) was done. Spectra were

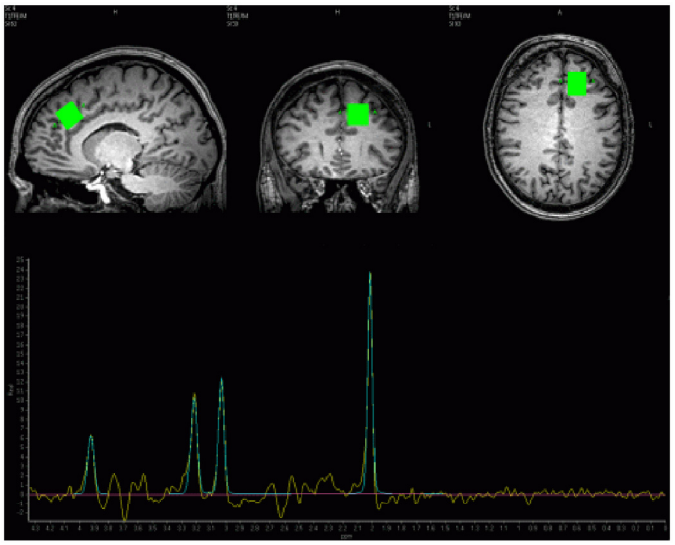

Figure 1. Representative illustration of voxel placement in the left prefrontal cortex (radiological display) and underneath a representative resulting spectrum.

recorded with the following parameters: TE = 144 ms, TR = 2000 ms, VOI = 20 × 20 × 20 mm, signal averages (NSA) = 128.

Data analyses

Differences in demographic characteristics and baseline data between the two treatment groups – in which brain metabolite measures were available – were analyzed with independent t-tests for numeral and chi-square tests for nominal variables. A mixed effects linear model was used to analyze differences in negative symptoms between the two groups. We included the baseline scores as a covariate to correct for potential differences at baseline.

To analyze the spectral data, LCModel with LCMgui was used [26]. To determine absolute metabolite concentrations, scaling based on the unsuppressed water peak was applied. Next, we calculated the association between the ratio of gray matter and white matter and the baseline NAA and Glx concentrations. As there was no significant correlation between the ratio of gray and white matter on the one hand and baseline Glx and NAA concentration on the other hand, we did not correct for partial volumes in our analysis to spare degrees of freedom. To study the association between treatment condition (active rTMS versus sham rTMS) and changes in concentration of Glx and NAA, we applied a linear regression model. To correct for differences at baseline, we included baseline Glx or NAA measures and an interaction term (treatment condition multiplied by baseline measure of Glx or NAA) as independent variables. Post-treatment measures of Glx or NAA were used as dependent variable.

In addition, correlation coefficients between changes in levels of Glx and NAA on the one hand and changes in negative symptoms on the other hand were calculated. Results were investigated at a p-value of 0.05. All analyses were conducted with IBM SPSS 20 (IBM SPSS Statistics 20.0, IBM Corp., Armonk, NY).

Table 1
Demographic and baseline clinical characteristics.

	Real TMS ($n = 11$)	Sham TMS ($n = 13$)	Significance
Age (years)	39.4 (11.6)	32.6 (9.9)	0.14
Sex (m/f)	9/2	10/3	0.77
Education (Verhage)	4.8 (1.8)	5.5 (1.2)	0.31
Age of onset	23.4 (4.2)	23.5 (5.7)	0.93
Illness duration (in years)	16 (11.0)	9.1 (8.3)	0.09
Type of medication			
Clozapine	3	5	
Olanzapine	3	2	
Risperidone	2	2	
Paliperidone	1	-	
Aripiprazole	2	3	
Quetiapine	1	-	
Haloperidol	1	-	
Other classical	3	3	
Polypharmacy	5	2	
Motor threshold*	59.7	Not determined	
SANS	54.5 (16.3)	42.9 (17.3)	0.11
PANSS Negative	19.7 (3.3)	19.4 (5.7)	0.86
PANSS Positive	11.7 (3.5)	12.3 (4.5)	0.73
PANSS General Psychopathology	32.1 (7.9)	28.8 (5.2)	0.23

Data are mean (±SD) or number of patients.
m, male; f, female; SANS, Schedule for the Assessment of Negative Symptoms; PANSS, Positive and Negative Syndrome Scale.
* Data of 1 subject are missing.

Results

Demographic and clinical characteristics

Table 1 shows the demographic and baseline clinical characteristics of the 24 patients. A total of 11 patients received active rTMS treatment and 13 patients received sham treatment. No significant differences in baseline symptomatology between the two groups were found. The mean illness duration was longer in the real rTMS group (trend level, $p = 0.09$). Differences in values of baseline Glx and NAA between the two groups were not statistically significant ($p = 0.1$ and $p = 0.26$, respectively, see Table 2). As we mentioned in the Materials and methods section, we corrected for differences at baseline by including baseline Glx and NAA measures in our analyses.

Negative symptoms

In contrast to our main report on the larger sample [27], for the groups for which we had pre- and post MRS measurements no significant changes were observed in negative symptoms as measured with the Scale for the Assessment of Negative Symptoms (SANS) ($F = 1.08$, $p = 0.31$), or with the PANSS negative symptoms subscale ($F = 0.35$, $p = 0.56$) in the comparison of the treatment group versus

Table 2
Changes in glutamate/glutamine (Glx) and N-Acetyl Aspartate (NAA) concentration in the left dorsolateral prefrontal cortex of patients with negative symptoms of schizophrenia treated with active prefrontal repetitive transcranial magnetic stimulation as compared to sham treatment.

	Groups	Baseline	End of treatment	Percentage change	p-value group difference at baseline
Glx (mM)	Active (n=11)	9.14 (2.16)	9.62 (1.89)	5.25	0.1
	Sham (n=13)	10.28 (0.99)	9.60 (1.13)	–6.61	
NAA (mM)	Active (n=11)	21.58 (4.19)	21.91 (3.57)	1.53	0.26
	Sham (n=13)	23.11 (2.12)	23.65 (2.08)	2.34	

Data are means (+/– SD), presented for the two treatment groups.

Table 3
Results of the linear regression analysis assessing the associations of condition (active rTMS treatment versus sham rTMS treatment) on glutamate/glutamine (Glx, neurotransmitter and precursor) and N-Acetyl Aspartate (NAA) concentration in the left prefrontal cortex of patients with schizophrenia.

Glx		**Dependent variable (post-treatment measurements of Glx)**		
	Independent variables	β-coefficient (95% confidence interval)	Standard error	*P*-value
	Condition (active versus sham)	–8.51 (–16.19 to –0.83)	3.68	0.032
	Glx baseline	–0.17 (–0.85 to 0.51)	0.32	0.61
	Interaction term (treatment condition × baseline measure Glx)	0.91 (0.16 to 1.67)	0.36	0.021
	Constant	11.34 (4.36 to 18.33)	3.35	0.003
NAA		**Dependent variable (post-treatment measurements of NAA)**		
	Independent variables	β-coefficient (95% confidence interval)	Standard error	*P*-value
	Condition (active versus sham)	–9.22 (–22.45 to 4)	6.34	0.16
	NAA baseline	0.41 (–0.099 to 0.91)	0.24	0.11
	Interaction term (treatment condition × baseline measure NAA)	0.38 (–0.2 to 0.95)	0.28	0.19
	Constant	14.28 (2.57 to 25.99)	5.61	0.02

the sham-stimulation group. This may have been due to lower statistical power. In addition, the patients that could not be included in the study had higher scores on the PANSS negative subscale (in the real rTMS group a mean score of 22.6 versus 19.7 and in the sham rTMS group a score of 21 versus 19.4) and the SANS (in the real rTMS group a mean score of 61 versus 55 and in the sham rTMS group a score of 52 versus 43). Furthermore, the subgroup of patients who received the active treatment and could not be included had a higher mean age (mean age of 47 versus 39) and showed less use of antipsychotic polypharmacy (percentage of patients using polypharmacy was 20% versus 45%).

The blinding process was successful. In the treatment group 64% thought they had received the active treatment, and in the sham group 69% ($Chi^2 = 0.54$).

MRS data

Table 2 shows the Glx and NAA concentrations before and after rTMS treatment in both groups and the percentages of change. Table 3 shows the results of the linear regression analyses.

The linear regression analyses, which corrected for differences at baseline, found significant associations between changes in Glx and treatment condition (β –8.51 [95% CI –16.19 to –0.8], $p = 0.032$) and the interaction term (β 0.91 [95% CI 0.16 to 1.67], $p = 0.021$). No significant associations between changes in NAA and treatment condition (β –9.22 [95% CI –22.45 to 4], $p = 0.16$) and the interaction term (β 0.38 [95% CI –0.2 to 0.95], $p = 0.19$) were found. Although there was an increase of Glx in treatment group, there was no significant correlation between changes in levels of Glx and NAA on the one hand and changes in negative symptoms on the other hand. However, sample size was small.

In addition, we observed that in the rTMS treatment group those patients using antipsychotic monotherapy ($n = 6$) all showed an increase of Glx after rTMS treatment (mean increase = 1.05 mM), whereas the Glx concentration in patients using antipsychotic polypharmacy ($n = 5$) on average showed a slight decrease after rTMS treatment (mean decrease = –0.2 mM), see Table 4. Antipsychotic polypharmacy combinations included a combination of clozapine and a typical antipsychotic ($n = 3$) or a combination of two atypical antipsychotics ($n = 2$). In the antipsychotic monotherapy group five patients were taking atypical antipsychotics and one patient was taking a typical antipsychotic. D_2 receptor occupancy [28] in the group of patients using antipsychotic polypharmacy was higher (85%) as compared to the group of patients using antipsychotic monotherapy (74%).

Discussion

To our knowledge, this is the first study reporting on the effects of a 10 Hz rTMS treatment on metabolite concentrations in the prefrontal area of the brain of patients with schizophrenia. In our study, we found a significant association between changes in Glx in the left DLPFC and treatment condition in patients with negative symptoms of schizophrenia receiving active or sham treatment. We observed an increase of Glx concentration in the active treatment group and a decrease of Glx concentration in the group receiving sham treatment. Our results are in agreement with the findings of earlier studies that reported an increase of Glx after prefrontal rTMS in healthy volunteers [17] and in patients with depression [18]. It is important to note that our fMRI study, which was also conducted among a subsample of the same study, found an increase in task-related brain activity in the right DLPFC and the right medial frontal gyrus in the active treatment group as compared to sham treatment [29]. The increase of Glx concentration in the active treatment group supports the rationale of prefrontal high frequency rTMS treatment for negative symptoms, namely that it increases prefrontal metabolism.

The subsample of our ^{1}H-MRS study was part of a larger randomized controlled trial that found a positive treatment response [27]. However, in the ^{1}H-MRS subsample no significant improvement of negative symptoms was found. Thus, the observed association between Glx concentration and treatment condition was not accompanied by a clinical improvement in the active treatment group. However, the negative findings may be due to low statistical power, considering the relatively small sample size of the subgroup. In addition, a large percentage of the patients (45%) in the rTMS treatment group was using antipsychotic polypharmacy. In this sample of patients using antipsychotic polypharmacy the Glx

Table 4
The influence of antipsychotic monotherapy versus antipsychotic polypharmacy on changes in glutamate/glutamine (Glx) concentration in the left dorsolateral prefrontal cortex of patients with negative symptoms of schizophrenia treated with active or sham prefrontal repetitive transcranial magnetic stimulation.

Condition		Baseline Glx	Post-treatment Glx	Mean change
Active treatment	Antipsychotic monotherapy ($n = 6$)	9.51 (1.27)	10.56 (0.69)	1.05 mM
	Antipsychotic polypharmacy ($n = 5$)	8.69 (3.04)	8.49 (2.33)	–0.2 mM
Sham treatment	Antipsychotic monotherapy ($n = 11$)	10.39 (1.05)	9.68 (1.21)	–0.71 mM
	Antipsychotic polypharmacy ($n = 2$)	9.7 (0.16)	9.13 (0.5)	–0.57 mM

Data are means (±SD).

did not increase after rTMS treatment. Indeed, all antipsychotics block dopamine and some also act on serotonin receptors. Therefore they may interfere with the mechanism of prefrontal rTMS, namely by modulating brain metabolism in the prefrontal cortex, the anterior cingulate cortex, the limbic lobe and the striatum [19,30–33]. Increase in Glx did occur in all patients ($n = 6$) using antipsychotic monotherapy in the treatment group.

In our study, the measurement of Glx and NAA was restricted to the left DLPFC due to scan time constraints. Indeed, most studies investigating rTMS for negative symptoms have targeted the left DLPFC [12,13,15] because of its putative role in the pathophysiology of negative symptoms of schizophrenia [34,35]. It would have been informative to also investigate Glx and NAA levels in the right DLPFC, as we also stimulated the right DLPFC. In addition, rTMS may modulate brain metabolism in other connected areas, such as the anterior cingulate cortex and the orbitofrontal cortex, which have also been implicated in negative symptoms [36,37]. In a study in patients with a depressive disorder, an increase of NAA in the anterior cingulate after rTMS was found [19]. In our study rTMS treatment did not influence NAA concentration in the left DLPFC. Future studies may benefit by also investigating areas connected to the DLPFC.

A limitation of the current study is that changes in dopamine concentration cannot be determined using ^{1}H-MRS, as several studies have found rTMS treatment induces changes in the dopamine system [30,38,39]. Other limitations include the small sample size, the fact that the trained nurses administering the rTMS treatment were not blinded, the measure of Glx (a sum of glutamate and glutamine) instead of glutamate proper, the mode of sham stimulation (which may induce a small amount of voltage in the brain), the relatively long echo time of 144 ms which is less suitable to detect changes in Glx and the baseline differences in illness duration of the treatment groups.

In conclusion, in our study bilateral 10 Hz rTMS treatment of the DLPFC in patients with schizophrenia suffering from predominant negative symptoms influenced Glx concentration in the left DLPFC of patients with schizophrenia, but not NAA concentration. Larger studies, including PET investigations of (changes in) dopamine concentration, are needed to further investigate the underlying neuronal mechanisms of rTMS treatment in patients with schizophrenia.

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References

[1] Milev P, Ho BC, Arndt S, Andreasen NC. Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. Am J Psychiatry 2005;162:495–506.
[2] Murphy BP, Chung YC, Park TW, McGorry PD. Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. Schizophr Res 2006;88:5–25.
[3] Wolkin A, Sanfilipo M, Wolf AP, Angrist B, Brodie JD, Rotrosen J. Negative symptoms and hypofrontality in chronic schizophrenia. Arch Gen Psychiatry 1992;49:959–65.
[4] Gonul AS, Kula M, Esel E, Tutus A, Sofuoglu S. A Tc-99m HMPAO SPECT study of regional cerebral blood flow in drug-free schizophrenic patients with deficit and non-deficit syndrome. Psychiatry Res 2003;123:199–205.
[5] Callicott JH, Bertolino A, Egan MF, Mattay VS, Langheim FJ, Weinberger DR. Selective relationship between prefrontal N-acetylaspartate measures and negative symptoms in schizophrenia. Am J Psychiatry 2000;157:1646–51.
[6] Tanaka Y, Obata T, Sassa T, Yoshitome E, Asai Y, Ikehira H, et al. Quantitative magnetic resonance spectroscopy of schizophrenia: relationship between decreased N-acetylaspartate and frontal lobe dysfunction. Psychiatry Clin Neurosci 2006;60:365–72.
[7] Sigmundsson T, Maier M, Toone BK, Williams SC, Simmons A, Greenwood K, et al. Frontal lobe N-acetylaspartate correlates with psychopathology in schizophrenia: a proton magnetic resonance spectroscopy study. Schizophr Res 2003;64:63–71.
[8] Smesny S, Gussew A, Biesel NJ, Schack S, Walther M, Rzanny R, et al. Glutamatergic dysfunction linked to energy and membrane lipid metabolism in frontal and anterior cingulate cortices of never treated first-episode schizophrenia patients. Schizophr Res 2015;168:322–9.
[9] Aleman A. Use of repetitive transcranial magnetic stimulation for treatment in psychiatry. Clin Psychopharmacol Neurosci 2013;11:53–9.
[10] Freitas C, Fregni F, Pascual-Leone A. Meta-analysis of the effects of repetitive transcranial magnetic stimulation (rTMS) on negative and positive symptoms in schizophrenia. Schizophr Res 2009;108:11–24.
[11] Slotema CW, Blom JD, Hoek HW, Sommer IE. Should we expand the toolbox of psychiatric treatment methods to include repetitive transcranial magnetic stimulation (rTMS)? A meta-analysis of the efficacy of rTMS in psychiatric disorders. J Clin Psychiatry 2010;71:873–84.
[12] Dlabac-de Lange JJ, Knegtering R, Aleman A. Repetitive transcranial magnetic stimulation for negative symptoms of schizophrenia: review and meta-analysis. J Clin Psychiatry 2010;71:411–18.
[13] Shi C, Yu X, Cheung EF, Shum DH, Chan RC. Revisiting the therapeutic effect of rTMS on negative symptoms in schizophrenia: a meta-analysis. Psychiatry Res 2014;215:505–13.
[14] Zhao S, Kong J, Li S, Tong Z, Yang C, Zhong H. Randomized controlled trial of four protocols of repetitive transcranial magnetic stimulation for treating the negative symptoms of schizophrenia. Shanghai Arch Psychiatry 2014;26:15–21.
[15] Wobrock T, Guse B, Cordes J, Wolwer W, Winterer G, Gaebel W, et al. Left prefrontal high-frequency repetitive transcranial magnetic stimulation for the treatment of schizophrenia with predominant negative symptoms: a sham-controlled, randomized multicenter trial. Biol Psychiatry 2015;77:979–88.
[16] Hasan A, Guse B, Cordes J, Wolwer W, Winterer G, Gaebel W, et al. Cognitive effects of high-frequency rTMS in schizophrenia patients with predominant negative symptoms: results from a multicenter randomized sham-controlled trial. Schizophr Bull 2015;42:608–18.
[17] Michael N, Gosling M, Reutemann M, Kersting A, Heindel W, Arolt V, et al. Metabolic changes after repetitive transcranial magnetic stimulation (rTMS) of the left prefrontal cortex: a sham-controlled proton magnetic resonance spectroscopy (1H MRS) study of healthy brain. Eur J Neurosci 2003;17:2462–8.
[18] Luborzewski A, Schubert F, Seifert F, Danker-Hopfe H, Brakemeier EL, Schlattmann P, et al. Metabolic alterations in the dorsolateral prefrontal cortex after treatment with high-frequency repetitive transcranial magnetic stimulation in patients with unipolar major depression. J Psychiatr Res 2007;41:606–15.
[19] Zheng H, Jia F, Guo G, Quan D, Li G, Wu H, et al. Abnormal anterior cingulate N-acetylaspartate and executive functioning in treatment-resistant depression after rTMS therapy. Int J Neuropsychopharmacol 2015;18.
[20] Dlabac-de Lange JJ, Bais L, van Es FD, Visser BG, Reinink E, Bakker B, et al. Efficacy of bilateral repetitive transcranial magnetic stimulation for negative symptoms of schizophrenia: results of a multicenter double-blind randomized controlled trial. Psychol Med 2014;1–13.
[21] Giel R, Nienhuis FJ. SCAN-2.1: Schedules for Clinical Assessment in Neuropsychiatry [in Dutch]. Geneva/Groningen: WHO, 1996.
[22] Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261–76.
[23] Schutter DJ, van Honk J. A standardized motor threshold estimation procedure for transcranial magnetic stimulation research. J ECT 2006;22:176–8.
[24] Beam W, Borckardt JJ, Reeves ST, George MS. An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. Brain Stimul 2009;2:50–4.
[25] Andreasen NC. Negative symptoms in schizophrenia. Definition and reliability. Arch Gen Psychiatry 1982;39:784–8.
[26] Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30:672–9.
[27] Dlabac-de Lange JJ, Bais L, van Es FD, Visser BG, Reinink E, Bakker B, et al. Efficacy of bilateral repetitive transcranial magnetic stimulation for negative symptoms of schizophrenia: results of a multicenter double-blind randomized controlled trial. Psychol Med 2015;45:1263–75.
[28] Lako IM, van den Heuvel ER, Knegtering H, Bruggeman R, Taxis K. Estimating dopamine D(2) receptor occupancy for doses of 8 antipsychotics: a meta-analysis. J Clin Psychopharmacol 2013;33:675–81.
[29] Dlabac-de Lange JJ, Liemburg EJ, Bais L, Renken RJ, Knegtering H, Aleman A. Effect of rTMS on brain activation in schizophrenia with negative symptoms: a proof-of-principle study. Schizophr Res 2015;168:475–82.
[30] Strafella AP, Paus T, Barrett J, Dagher A. Repetitive transcranial magnetic stimulation of the human prefrontal cortex induces dopamine release in the caudate nucleus. J Neurosci 2001;21:RC157.

[31] Sibon I, Strafella AP, Gravel P, Ko JH, Booij L, Soucy JP, et al. Acute prefrontal cortex TMS in healthy volunteers: effects on brain 11C-alphaMtrp trapping. Neuroimage 2007;34:1658–64.
[32] Kole MH, Fuchs E, Ziemann U, Paulus W, Ebert U. Changes in 5-HT1A and NMDA binding sites by a single rapid transcranial magnetic stimulation procedure in rats. Brain Res 1999;826:309–12.
[33] Ben-Shachar D, Gazawi H, Riboyad-Levin J, Klein E. Chronic repetitive transcranial magnetic stimulation alters beta-adrenergic and 5-HT2 receptor characteristics in rat brain. Brain Res 1999;816:78–83.
[34] Hovington CL, Lepage M. Neurocognition and neuroimaging of persistent negative symptoms of schizophrenia. Expert Rev Neurother 2012;12:53–69.
[35] Hill K, Mann L, Laws KR, Stephenson CM, Nimmo-Smith I, McKenna PJ. Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies. Acta Psychiatr Scand 2004;110:243–56.
[36] Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, et al. Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: a DTI study. Schizophr Res 2014;157:190–7.
[37] Nelson BD, Bjorkquist OA, Olsen EK, Herbener ES. Schizophrenia symptom and functional correlates of anterior cingulate cortex activation to emotion stimuli: an fMRI investigation. Psychiatry Res 2015;234:285–91.
[38] Keck ME, Welt T, Muller MB, Erhardt A, Ohl F, Toschi N, et al. Repetitive transcranial magnetic stimulation increases the release of dopamine in the mesolimbic and mesostriatal system. Neuropharmacology 2002;43:101–9.
[39] Cho SS, Strafella AP. rTMS of the left dorsolateral prefrontal cortex modulates dopamine release in the ipsilateral anterior cingulate cortex and orbitofrontal cortex. PLoS ONE 2009;4:e6725.