PREDICTING RESPONSE TO rTMS TREATMENT IN PATIENTS WITH SCHIZOPHRENIA FROM BRAIN MORPHOLOGY

Chapter 6

Claire Kos¹
Leonie Bais¹
Jan-Bernard C. Marsman¹
Sanne Koops²,³
Jozarni Dlabac-de Lange¹,⁵
Henderikus Kneegtering⁴,⁵
Iris E. Sommer²,³
Marie-José van Tol¹
André Aleman¹,⁵,⁶

¹ Department of neuroscience, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan 2, 9713 AW, The Netherlands
² Department of Psychiatry, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands
³ UMC Utrecht Brain Center Rudolf Magnus, Universiteitsweg 100 Utrecht, 3584 CG, Utrecht, The Netherlands
⁴ Lentis Research, Lentis Center for Mental Health Care, Hereweg 80, 9725 AG, Groningen, The Netherlands
⁵ Rob Giel Research Center, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands
⁶ Department of Psychology, University of Groningen, Grote Kruisstraat 2/1, 9712 TS, Groningen, The Netherlands

Tantalizing city
Abstract
The use of repetitive Transcranial Magnetic Stimulation (rTMS) as a treatment option for patients with schizophrenia has gained interest. However, large inter-individual differences in response to treatment have been observed, suggesting that patient-specific characteristics play a role in treatment efficacy. Reliable predictors of treatment efficacy in patients with schizophrenia have not yet been established. The objective of the study was to evaluate whether morphological factors, including scalp-to-cortex distance (SCD) and gray matter density (GMD) of the stimulated brain region would predict response to rTMS treatment in patients with schizophrenia suffering from auditory verbal hallucinations (AVH) or persistent negative symptoms. For this purpose, five clinical trials were combined, resulting in a dataset of 68 patients with schizophrenia and related psychotic disorders. All patients underwent rTMS treatment and Magnetic Resonance Imaging. Correlation coefficient calculations and hierarchical linear regression models were applied to investigate the relationship between treatment efficacy and SCD and GMD of the stimulated brain region (either the temporo-parietal junction [TPJ] or dorsolateral prefrontal cortex [DLPFC]). The results showed that shorter SCD and lower GMD of the TPJ was associated with greater AVH improvement after rTMS treatment. However, no relationship was observed between GMD and SCD of the DLPFC and negative symptom improvement. When the data was pooled for the AVH and negative symptom trials, all associations were non-significant. It could be concluded that SCD and GMD are possible predictors of treatment success, especially for trials targeting the TPJ aimed at alleviating AVH. When proved to be robust, such information can ultimately guide patient selection or adjustments in stimulation parameters for rTMS treatment.

Keywords: rTMS, schizophrenia, treatment prediction, scalp-to-cortex distance, gray matter density

Introduction
Over the last decade, the use of repetitive Transcranial Magnetic Stimulation (rTMS) for the treatment of symptoms of schizophrenia has gained interest. This non-invasive technique makes use of a TMS coil placed on the scalp, producing a rapidly changing magnetic field which induces an electrical current in the underlying cortex (Barker, Jalinous, & Freeston, 1985). Depending on the applied frequency, it is possible to depolarize (Chen et al., 1997; Wassermann, Wedegaertner, Ziemann, George, & Chen, 1998) or hyperpolarize neurons (Cohen et al., 1999; Peinemann et al., 2004). In patients with persistent auditory verbal hallucinations (AVH), the temporo-parietal junction (TPJ) area has shown elevated levels of activation (Jardri, Pouchet, Pins, & Thomas, 2011). Hence, by targeting the TPJ area with low frequency rTMS, AVH could theoretically be reduced (Hoffman et al., 1999). Indeed, meta-analyses have shown efficacy of this paradigm as compared to double blind matched placebo treatment (Slotema, Aleman, Daskalakis, & Sommer, 2012). Negative symptoms on the other hand, have been related to lower involvement of the dorsolateral prefrontal cortex (Potkin et al., 2002; Wolkin et al., 1992). With the use of high frequency rTMS over the dorsolateral prefrontal cortex, activation in this region is expected to increase, which may yield in a reduction of negative symptoms (DLPFC, Dlabac-de Lange, Knegtering, & Aleman, 2010; Shi, Yu, Cheung, Shum, & Chan, 2014). Overall, however, results from studies that investigated the efficacy of rTMS for negative symptoms in schizophrenia are inconclusive (Cole, Green Bernacki, Helmer, Pinninti, & O’reardon, 2015; for meta-analyses see Dlabac-de Lange et al., 2010; Freitas, Fregni, & Pascual-Le-
one, 2009; Fusar-Poli et al., 2015; Y. Zhang et al., 2013). Also within studies, large inter-individual differences in response to rTMS treatment have been observed (Bais et al., 2014), which may be the result of variability in patient-specific characteristics. It is therefore of relevance to identify the factors that are associated with response to rTMS treatment. In this context, demographical and clinical factors have been suggested to affect treatment success, but possibly also morphological and neurophysiological factors are involved (Silverstein et al., 2015). Identifying biomarkers predictive of response to treatment in the schizophrenia population would be useful in selecting patients for treatment or to adjust treatment parameters on individual characteristics.

In patients with depression, greater scalp-to-cortex distance has been associated with lower anti-depressive effects of rTMS (Kozel et al., 2000; Mosimann et al., 2002; Nahas et al., 2001). Given the decay of the induced electrical field with distance, greater scalp-to-cortex distance is likely to be associated with less electromagnetic stimulation of cortical tissue, i.e. reduced likelihood of a depolarizing effect on neurons (Bohning, 2000). Furthermore, gray matter density and cortical thickness levels of the stimulated area may modulate effects of rTMS, as they have been associated with cortical excitability in healthy individuals (Conde et al., 2012; List et al., 2013). In patients with schizophrenia, factors related to rTMS response are however less well established compared to patients with depressive disorders. Only one study published in 2015 was identified, by Nathou et al. (2015), which demonstrated that beneficial treatment effects of rTMS for patients with schizophrenia and AVH were predicted by lower scalp-to-cortex distance and higher gray matter density of the stimulated area. Nathou et al. (2015) suggest that greater atrophy of the TPJ can increase scalp-to-cortex distance, possibly reducing treatment effects of rTMS. In patients with schizophrenia, reductions of gray matter volumes have also been observed in frontal regions (Gur et al., 2000; van Haren et al., 2011), which are possibly related to negative symptoms (Benoit, Bodnar, Malla, Joober, & Lepage, 2012). Like the TPJ area, frontal regions seem to decrease in volume over time (van Haren et al., 2011). However, to our knowledge the relationship between gray matter density and scalp-to-cortex distance in patients with negative symptoms who received prefrontal rTMS has not yet been studied.

When investigating possible associations between morphological factors of the stimulated regions and treatment response, it is also important to take the morphological factors of the primary motor cortex into account. rTMS stimulation intensity is typically determined at the level of the primary motor cortex, yet, the gray matter of this region may not be similarly affected as in temporal and prefrontal regions (Chan et al., 2009; van Haren et al., 2011). It is thus conceivable that the level of excitability of the stimulated regions does not accord with that of the primary motor cortex, and that the strength of stimulation is therefore suboptimal. The inconclusive results on this matter warrant further study.

The aim of the present study was to investigate whether scalp-to-cortex distance and gray matter density are associated with response to rTMS treatment in patients with schizophrenia. These morphological factors were determined in a final composite treatment sample consisting of 68 individual patients, pooled from previously published rTMS trials. A subsample of patients received rTMS for the treatment of AVH on the left TPJ, bilateral TPJ, or a region of maximal hallucinatory activation as determined with fMRI (in the left or right temporal or parietal cortex). Another subsample of patients was treated with rTMS of the bilateral DLPFC targeted at reducing persistent negative
symptoms. We aimed to associate the degree of treatment response with the measures of gray matter density and scalp-to-cortex distance of the stimulated regions, as well as the primary motor cortex. Following Nathou et al. (2015), we hypothesized that a better treatment response is predicted by greater gray matter density and smaller scalp-to-cortex distance of the stimulated regions.

**Methods**

**Participants**

For the purpose of this study, data was available for 79 participants from five separate clinical trials, three of which were performed by the University Medical Center Utrecht (de Weijer et al., 2014; Koops et al., 2016, registered at clinicaltrials.gov, number NCT01512290; Slotema et al., 2011, registered at the Dutch Trial Register, number NTR2012) and two by the University Medical Center Groningen (Bais et al., 2014; Dlabac-de Lange et al., 2015, registered in the Dutch trial register, trial numbers NTR1813 and NTR1261, respectively). All trials investigated the effects of rTMS; four for the alleviation of auditory verbal hallucinations (Bais et al., 2014; de Weijer et al., 2014; Koops et al., 2016; Slotema et al., 2011), and one for negative symptoms (Dlabac-de Lange et al., 2015). In addition, these patients underwent an MRI-scan. Only patients that received real rTMS (compared to sham) were selected for the current study. Eight patients participated in multiple treatment trials. For these participants, data was included of the first trial they participated in, which was the trial by Slotema et al. (2011) in all cases, resulting in sample of 71 included participants that were considered for analyses. Study and rTMS stimulation parameters are specified in Tables 1 and 2. Demographical characteristics of the included patients are presented in Table 3.

Patients with a diagnosis of schizophrenia (Bais et al., 2014; de Weijer et al., 2014; Dlabac-de Lange et al., 2015; Koops et al., 2016; Slotema et al., 2011), schizophreniform disorder (Koops et al., 2016), schizoaffective disorder (Bais et al., 2014; de Weijer et al., 2014; Dlabac-de Lange et al., 2015; Koops et al., 2016; Slotema et al., 2011), or psychosis not otherwise specified (de Weijer et al., 2014; Koops et al., 2016; Slotema et al., 2011) were forwarded by their clinicians for participation. Diagnosis was confirmed by a trained interviewer, using the Schedules for Clinical Assessment in Neuropsychiatry ([SCAN] World Health Organization, 1999; Dlabac-de Lange et al., 2015) or Diagnostic and Statistical Manual of Mental Disorders, fourth edition ([DSM-IV] American Psychiatric Association, 1994; Bais et al., 2014; Slotema et al., 2011), or in context of clinical care as usual by a trained psychiatrist (de Weijer et al., 2014; Koops et al., 2016). Both inpatients and outpatients were included. All patients were over 18 years old and presented medication resistant, frequent AVH (Bais et al., 2014; Koops et al., 2016; Slotema et al., 2011), or persistent negative symptoms (minimum score of 15 on the negative symptoms subscale of the Positive and Negative Syndrome Scale, Kay, Fiszbein, & Opler, 1987; Dlabac-de Lange et al., 2015). Prior to inclusion, patients were stable on medication. During participation, medication had to remain unchanged until the last measurement. Patients were excluded in case of TMS or MRI contra-indications, presence of neurological or severe behavioral disorders, and previous (within the last six months) or current substance dependency (excluding nicotine dependency).

All patients provided oral and written consent after full explanation of the study. All studies were approved by an authorized local medical ethical committee, and in accordance with the latest version of the declaration of Helsinki (General Assembly of the World Medical Association, 2014).
Table 1. Study parameters of the included studies.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Location</th>
<th>Frequency</th>
<th>MT%</th>
<th>N sessions</th>
<th>N days</th>
<th>N pulses</th>
<th>Method for localization</th>
<th>Coil</th>
<th>Brand</th>
<th>TMS-machine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mono</td>
<td>TPJ (L, B)</td>
<td>1 Hz</td>
<td>90%</td>
<td>12</td>
<td>14400</td>
<td>10-20 EEG</td>
<td>70 mm figure-of-eight coil</td>
<td>Magstim</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mono</td>
<td>TPJ (L)</td>
<td>50 Hz</td>
<td>80%*</td>
<td>10</td>
<td>9000</td>
<td>10-20 EEG</td>
<td>70 mm figure-of-eight coil</td>
<td>Magstim</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Multi</td>
<td>TPJ (L) IMRI-guided</td>
<td>1 Hz</td>
<td>90%</td>
<td>15</td>
<td>18000</td>
<td>10-20 EEG &amp; Neuronavigation</td>
<td>70 mm figure-of-eight coil</td>
<td>Magstim</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Multi</td>
<td>IMRI-guided</td>
<td>1 Hz</td>
<td>90%</td>
<td>8</td>
<td>9600</td>
<td>70 mm figure-of-eight coil</td>
<td>Neuronavigation</td>
<td>Magstim</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DLPFC (B)</td>
<td>10x10 Hz</td>
<td>90%</td>
<td>30</td>
<td>15</td>
<td>60000</td>
<td>10-20 EEG</td>
<td>75 mm figure-of-eight coil</td>
<td>Medtronic</td>
<td></td>
</tr>
</tbody>
</table>

AHRS = Auditory Hallucination Rating Scale; AVH = Auditory Verbal Hallucinations; B-DLPFC = Stimulation of the bilateral dorsolateral prefrontal cortex; B-TPJ = Stimulation of the bilateral temporo-parietal junction; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, fourth edition; fMRI-guided = stimulated on location of maximal activation measured with functional Magnetic Resonance Imaging; L-TPJ = Stimulation of the left temporo-parietal junction; N = number of participants; P-DLPFC = Placebo stimulation of the bilateral dorsolateral prefrontal cortex; P-TPJ = Placebo stimulation of the left temporo-parietal junction; PANSS = Positive and Negative Syndrome Scale; PNS = Psychosis Not Otherwise Specified; PSYRATS = Psychotic Symptom Rating Scale; SA = Schizoaffective disorder; SANS = Scale for the Assessment of Negative Symptoms; SCAN = Schedules for Clinical Assessment in Neuropsychiatry; SP = Schizophreniform; SZ = Schizophrenia.

1 Bais et al. 2014
2 Koops et al. 2016
3 Skotema et al. 2011
4 De Weijer et al. 2014
5 Dlabac-de Lange et al. 2015

Table 2. Stimulation parameters of the included studies.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Location</th>
<th>Frequency</th>
<th>MT%</th>
<th>N sessions</th>
<th>N days</th>
<th>N pulses</th>
<th>Method for localization</th>
<th>Coil</th>
<th>Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TPJ (L, B)</td>
<td>1 Hz</td>
<td>90%</td>
<td>12</td>
<td>6 consecutive week days</td>
<td>14400</td>
<td>10-20 EEG</td>
<td>70 mm figure-of-eight coil</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TPJ (L)</td>
<td>50 Hz</td>
<td>80%</td>
<td>10</td>
<td>5 consecutive week days</td>
<td>9000</td>
<td>10-20 EEG</td>
<td>70 mm figure-of-eight coil</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TPJ (L) IMRI-guided</td>
<td>1 Hz</td>
<td>90%</td>
<td>15</td>
<td>15 consecutive week days</td>
<td>18000</td>
<td>10-20 EEG &amp; Neuronavigation</td>
<td>70 mm figure-of-eight coil</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>IMRI-guided</td>
<td>1 Hz</td>
<td>90%</td>
<td>8</td>
<td>5 consecutive work days then 3 weeks once a week</td>
<td>9600</td>
<td>70 mm figure-of-eight coil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DLPFC (B)</td>
<td>10x10 Hz</td>
<td>90%</td>
<td>30</td>
<td>15 consecutive week days</td>
<td>60000</td>
<td>10-20 EEG</td>
<td>75 mm figure-of-eight coil</td>
<td></td>
</tr>
</tbody>
</table>

*Max 51% of stimulator output; %MT = percentage of motor threshold used for stimulation; B = bilateral; DLPFC = Dorsolateral Prefrontal Cortex; fMRI-guided = stimulated on location of maximal activation measured with functional Magnetic Resonance Imaging L = left; TPJ = Temporo-Parietal Junction.

1 Bais et al. 2014
2 Koops et al. 2016
3 Skotema et al. 2011
4 De Weijer et al. 2014
5 Dlabac-de Lange et al. 2015
Treatment protocols of the five included trials (see also Table 2)

**Auditory verbal hallucinations trial 1 (Bais et al., 2014), n=21**

Patients received two rTMS sessions per day (one in the morning and one in the afternoon), for six consecutive working days. A Magstim Rapid System (Magstim Company Ltd, Whitland, Wales) with a 70-mm figure-of-eight coil was used for the administration of the TMS pulses to the TPJ. Stimulation was provided to the left (N=16) or bilateral TPJ (N=15). The stimulation site was determined using the 10-20 International System; in the middle of the T3 and P3 electrode positions for left stimulation and in the middle of T4 and P4 for right stimulation. A template was used to position the center of the coil over the marked area. All participants received twelve 1 Hz rTMS stimulation sessions of 20 minutes per session, with 10 minutes left and 10 minutes right stimulation in the bilateral condition, with a total of 14,400 pulses during the treatment course. The stimulation was set at 90% of the individually determined motor threshold. The motor threshold was defined as the minimum required intensity to provoke a visible movement in the dominant hand in five out of 10 magnetic pulses that were administered at the primary motor cortex (Schutter & van Honk, 2006). Motor threshold data was available for 31 participants, and MR-data for 21 participants (of which 10 received active stimulation on the left TPJ, and 11 on the bilateral TPJ).

**Auditory verbal hallucinations trial 2 (Koops et al., 2016), n=8**

Patients received 10 continuous theta burst stimulation sessions (based on Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). Treatment sessions were provided on five consecutive days, twice a day. Sessions were composed of 60-s stimulation trains of three pulses at 50 Hz, repeated every 200 ms, resulting in a 9000-pulses treatment course. Stimulation was targeted at the left TPJ, using the 10-20 International System (i.e. midway T3 and P3 electrode positions). A Magstim Rapid 2 (Magstim Company, Whitland, Wales) with a 70-mm figure-of-eight coil was used for rTMS treatment. Stimulation was provided at 80% of the individually determined motor threshold, according to the procedure described by Schutter & van Honk (2006). Of note, stimulation was maximally provided at 51% of the maximum stimulator output, resulting in three patients that received stimulation at 64-78% of the individual motor threshold (instead of stimulation at 80%). Motor threshold data was available for seven participants and MR-data for eight participants that received active rTMS treatment.

**Auditory verbal hallucinations trial 3 (Slotema et al., 2011), n=17**

On 15 consecutive working days, a treatment session of 1 Hz, for 20 minutes was provided, resulting in 18000 pulses over the entire treatment course. A Magstim Rapid 2 (Magstim Company, Whitland, Wales) with a 70-mm figure-of-eight coil was used for rTMS treatment. Participants received...
stimulation targeting the area of maximal hallucinatory activation, determined during functional MR-scanning (N=17), or at the left temporo-parietal junction, determined using the 10-20 International System; in the middle of the T3 and P3 electrode positions (N=17). Stimulation was provided at 90% of the individually determined motor threshold (Schutter & van Honk, 2006). Data was available for 17 participants that received active stimulation on the TPJ (as determined using the 10-20 International System). Data on the individually determined region of maximal hallucinatory activation were not available for the present study. Motor threshold data was available for 16 participants that received active stimulation of the TPJ.

Auditory verbal hallucinations trial 4 (de Weijer et al., 2014), n=10
Treatment was provided for five consecutive workdays, followed by one treatment session per week, for three weeks, resulting in eight sessions in total. Patients were randomized into either a 1 Hz treatment arm, or 20 Hz treatment arm. Within the first arm, patients received 20 minutes of stimulation at 1 Hz (9600 pulses), at 90% of the motor threshold (Schutter & van Honk, 2006). Patients in the second arm received 13 trains of 20 Hz, with an inter-train interval of 50 s (20800 pulses), at 80% of the motor threshold (Schutter & van Honk, 2006). Both groups received treatment with a 70-mm figure-of-eight coil at the brain region of individual hallucination-related activation measured using fMRI (all were located in the left or right temporal or parietal cortex). A template was used in order to provide treatment at the exact location. The study was not placebo-controlled. MR-data was available for 18 participants that received active rTMS treatment, however data of eight participants were not included, as they already participated in trial 3 by Slotema et al. (Slotema et al., 2011). Motor threshold data was not available.

Negative symptom trial (Dlabac-De Lange et al., 2015), n=15
Stimulation was provided in two sessions daily for 15 consecutive workdays. A Medtronic MagPro X100 stimulator (Medtronic, USA) with a 75-mm figure-of-eight coil was used for the administration of rTMS pulses to the bilateral dorsolateral prefrontal cortex (DLPFC). In the morning session, the left DLPFC was stimulated, and in the afternoon the right DLPFC. The site of stimulation corresponded with F3 and F4 electrodes of the 10-20 International System and the coil was placed using a template. All participants received 30 stimulation sessions of 20 minutes at 10 Hz, consisting of 20 trains of 10 s, with an inter-train interval of 50 s, adding up to 60,000 pulses per treatment course. Stimulation was provided at 90% of the individually determined motor threshold (Schutter & van Honk, 2006). Motor threshold data was available for 13 participants, and MR-data was available for 15 participants that received active rTMS treatment.

Clinical measures (see also Table 1)
Clinical assessments were performed at baseline, directly after rTMS treatment, and at one month follow-up (Bais et al., 2014; de Weijer et al., 2014; Dlabac-de Lange et al., 2015; Koops et al., 2016; Slotema et al., 2011), two months follow-up (Slotema et al., 2011), and three months follow-up (Bais et al., 2014; Dlabac-de Lange et al., 2015; Slotema et al., 2011). Primary outcome measures were the Auditory Hallucination Rating Scale ([AHRS] Hoffman et al., 2003)(Bais et al., 2014; de Weijer et al., 2014; Koops et al., 2016; Slotema et al., 2011), the Positive and Negative Syndrome Scale ([PANSS] Kay et al., 1987)(Bais et al., 2014; Dlabac-de Lange et al., 2015), Psychotic Symptom Rating Scale ([PSYRATS], Haddock, McCarron, Tarrier, & Faragher, 1999)(Koops et
al., 2016), and the Scale for the Assessment of Negative Symptoms ([SANS], Andreasen, 1984) (Dlabac-de Lange et al., 2015). The AHRS is a 7-item questionnaire that is frequently used to assess the severity of auditory hallucinations; the PANSS includes a 7-item positive, 7-item negative, and a 16-item general symptom subscale; the PSYRATS quantifies the severity of delusions (6 items) and auditory hallucinations (11 items); and lastly, the SANS is a 26-item rating instrument for the assessment of negative symptoms.

**Image acquisition**

Participants from the AVH trial of Bais et al. (2014) and the negative symptom trial of Dlabac-de Lange et al. (2015) were scanned in the same center, using a Philips Intera 3 Tesla MRI scanner (Best, the Netherlands) equipped with an 8-channel SENSE head coil. A three-dimensional T1 weighted anatomical image was obtained with the following parameters for the AVH trial and the negative symptom trial respectively: TR/TE = 25.00/4.6 ms; 9.00/3.5 ms, flip angle = 30/8 degrees, 160/170 slices; FOV (ap, fh, rl) = 256 × 160 × 204 / 232 x 170 x 256; slice thickness = 1 mm (no gap); voxel size: 1 × 1 × 1 mm. The participants from the AVH trials of Koops et al. (2016), Slotema et al. (2011) and De Weijer et al. (2014) were scanned in another center, using a Philips Achieva 3 Tesla Clinical Scanner, equipped with an 8-channel SENSE head coil (Best, the Netherlands). Anatomical images were obtained with the following parameters: TR/TE = 9.96/4.6 ms, flip angle = 8 degrees, 160 slices; FOV (ap, fh, rl) = 224 x 160 x 168, slice thickness = 1 mm (no gap), voxel size = .875 x .875 x 1. Two participants from the AVH trial of Koops et al. (2016) were scanned with a slightly different sequence: TR/TE = 10.03/4.6 ms, flip angle = 8 degrees, 200 slices; FOV (ap, fh, rl) = 240 x 240 x 160; slice thickness = 0.8 mm (no gap); voxel size: 0.75 x 0.75 x 0.75 mm.

**Preprocessing of anatomical images**

Before calculation of the scalp-to-cortex distance and gray matter density, we performed visual inspection of the anatomical images to check for possible artefacts. Further, image origins were manually set to the center point of the anterior commissure. Preprocessing steps included spatial normalization to Montreal Neurological Institute (MNI) space and segmentation of the images to identify gray matter, white matter, cerebrospinal fluid, skull, soft tissue outside the brain, and air or other non-brain related data. Imaging data for one participant from the AVH trial of De Weijer et al. (2014), and one participant from the negative symptoms trial of Dlabac-de Lange et al. (2015) were excluded due to excessive head movement. One additional participant from the AVH trial of Slotema et al. (2011) was excluded due to failed segmentation, leaving 68 participants for the analysis.

**Scalp-to-cortex distance**

The scalp-to-cortex distance (SCD) was calculated using the point of stimulation (for treatment and for determination of the motor threshold) on the scalp and the underlying gray matter at its nearest point. For the studies that used the 10-20 system for determination of the point of stimulation, we calculated the corresponding MNI coordinates. Using BrainStorm (Tadel, Baillet, Mosher, Pantazis, & Leahy, 2011), MNI coordinates for F3, F4, CP5 (point in between T3 and P3), CP6 (point in between T4 and P4), and C3 (primary motor cortex) were determined. First, around each stimulation point a sphere with a 5-mm radius was drawn using the Marsbar toolbox of the Statistical Parametric Mapping (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) software (Brett, Anton, Valabregue, & Poline, 2002), in MATLAB 2013a (The Mathworks, Natick MA, USA). Second, this
sphere in MNI space was transformed for each participant into native space using SPM12 (version 6470; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and in-house developed software. Third, this sphere in each individual anatomical image was dilated until the point where it reached gray matter, yielding the radius of the final (smallest) dilated sphere for determination of the absolute SCD in mm (Figure 1).

For patients in which neuro-navigation was applied, the target coordinates in native space were used as the center point of a 5-mm sphere. Because the stimulation coordinates were not located on the surface of the cortex, the point of stimulation on the scalp (in native space) was determined in a first step. A contour was drawn of the scalp, and the 5-mm sphere was dilated until it reached the contour. The coordinates of this point were used to complete the same procedure as described in the previous paragraph, including a dilation of a 5-mm sphere on the scalp until it reached the gray matter at its nearest point. In case of three participants, the initial dilation process failed, due to an artefact in the image of the scalp contour. This was corrected manually by minor editing of the segmented gray matter channel. For all participants, the spheres were visually checked by two authors (CK, LB).

Figure 1. The left panel presents a dilated sphere on the F3 location of an individual participant. The distance between the center point of the sphere and the underlying gray matter was used as a measure for SCD of the stimulated F3 location. The middle and right panels present the gray matter region-of-interest of an individual participant that was used for calculation of the GMD of F3.
Gray matter density

Gray matter density (GMD) was calculated for the region directly underneath the point of transcra- nial stimulation, for the treatment location as well as for the primary motor cortex. For this purpose, a binary sphere with a 5-mm radius was placed on the gray matter with its center at the point where the gray matter was closest to the point of stimulation on the scalp (as determined with the dilated sphere for scalp-to-cortex calculation, see Figure 1). This was done for each patient. These native ROIs were multiplied with the segmented gray matter of each participant using ImCalc in SPM12. Next, the average amount of gray matter per voxel within each sphere was calculated (GMD divided by the total number of voxels in the sphere) for each participant using in-house developed software. For all participants, the spheres on the gray matter were visually checked by two authors (CK, LB).

Total brain volume

For each person, the total brain volume (TBV) was calculated as the summation of total gray and white matter.

Statistical analysis

Data was analyzed using IBM SPSS Statistics (Version 23). In accordance with Nathou et al. (2015), we calculated ratio scores for the primary outcome measures of each study to evaluate treatment effects. Ratio scores were calculated for immediate treatment effects ($\Delta = (\text{Post} – \text{Pre}) / \text{Pre}$) using the primary outcome measures of each rTMS trial (see Table 4). Kendall’s Tau correlation coefficients were calculated for associations between ratio scores and SCD and GMD of the stimulated regions and TBV. For participants who received bilateral stimulation, the average SCD and GMD of the bilateral TPJ or DLPFC was calculated and used in further analyses.

For the main analysis, stepwise linear regression analyses using the hierarchical method were performed to evaluate possible predictors for rTMS treatment effects over all trials. The model included the ratio scores as dependent variable and the anatomical measures, SCD and GMD as independent variables in the first step. Furthermore, to control for differences in brain volume and age effects, TBV and age were included in the analysis in the second step. These stepwise regression analyses were repeated separately for studies targeting AVH and negative symptom dimensions; thus, one model including the ratio scores from all four AVH trials, and one model including the ratio scores from the trial on negative symptoms. Furthermore, analyses were repeated once more including only AVH trials that used the international 10-20 system to target the TPJ, while individual fMRI-guided rTMS was left out. The latter two analyses were executed in order to evaluate the effects for studies that targeted the same anatomical region (DLPFC vs. TPJ) and used the same method for localization (international 10-20 system).

An additional stepwise linear regression analysis was performed to evaluate if the motor threshold could be predicted by anatomical characteristics of the primary motor cortex. The model included the motor threshold as the dependent variable and SCD and GMD as independent variables in the first step. Furthermore, TBV and age were included in the analysis in the second step.

Finally, to compare the depth and density of the stimulated region and primary motor cortex, and to evaluate if these were related to each other, the SCD and GMD of the treatment locations and prima-
ry motor cortex were compared using a paired Student’s t-test. In addition, Kendall’s Tau correlation coefficients were calculated for associations between SCD and GMD of the treatment locations and primary motor cortex. The significance level for was set at \( p < .05 \) for all analyses. The reported p-values are two-tailed, except for the Kendall’s Tau correlation coefficients that were one-tailed (because of a priori hypotheses).

**Results**

**Target region**

Ratio scores for treatment efficacy, SCD, and GMD measures are presented per trial in Table 4 and Figure 2. Correlation coefficients for associations between these morphological measures and treatment efficacy can be found in Table 5. When the data was pooled for the AVH and negative symptom trials, all associations were non-significant. The hierarchical stepwise regression models on the pooled data also did not yield statistical significant predictors of treatment efficacy.

When the analyses were repeated for the AVH and negative symptom trials separately, SCD of the stimulated region was significantly associated with treatment efficacy in the AVH sample (\( r = .17, p = .046 \)). The stepwise regression model again did not yield significant predictors of treatment efficacy, although the model including SCD of the stimulated region as a predictor of treatment efficacy in AVH trials was significant at trend level (\( b = .03, t (49) = 1.82, p = .08 \)). The analyses were again repeated, now only including trials that determined locus of stimulation based on the international 10/20 system, thus excluding the fMRI-guided study (excluding de Weijer et al., 2014, leaving N=45). Simple correlation analyses revealed statistically significant correlations between treatment efficacy and SCD (\( r = .22, p = .02 \)) as well as GMD (\( r = .17, p = .05 \)). Furthermore, the hierarchical stepwise regression analyses revealed SCD as a significant predictor of treatment efficacy (\( b = .056, t (40) = 2.12, p = .04 \)). The model including SCD and GMD was trend wise significant, explaining 11.2 % of the variance (\( F(2,42) = 2.65, p = .08 \)). The analyses including only the negative symptom trial did not demonstrate statistical significant correlations or predictors of treatment efficacy.

**Table 4.** Anatomical characteristics of the included participants.

<table>
<thead>
<tr>
<th>Trial</th>
<th>( %\Delta )</th>
<th>SCD Target (m, SD)</th>
<th>SCD Motor (m, SD)</th>
<th>GMD Target (m, SD)</th>
<th>GMD Motor (m, SD)</th>
<th>TBV (m, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^1)</td>
<td>-.04</td>
<td>(1.22) 13.71</td>
<td>(1.42) 16.90</td>
<td>(.00088)</td>
<td>(.00064)</td>
<td>1169.44</td>
</tr>
<tr>
<td>2(^2)</td>
<td>.10</td>
<td>(1.22) 13.25</td>
<td>(.64) 15.88</td>
<td>(.00056)</td>
<td>(.00049)</td>
<td>1139.49</td>
</tr>
<tr>
<td>3(^3)</td>
<td>.15</td>
<td>(1.5) 13.13</td>
<td>(2.51) 16.81</td>
<td>(.00064)</td>
<td>(.00053)</td>
<td>1156.46</td>
</tr>
<tr>
<td>4(^4)</td>
<td>.12</td>
<td>(2.21) 16.22</td>
<td>(7.89) 17.11</td>
<td>(.00007)</td>
<td>(.00006)</td>
<td>1129.24</td>
</tr>
<tr>
<td>5(^5)</td>
<td>.12</td>
<td>(2.22) 17.86</td>
<td>(.00072)</td>
<td>(.00067)</td>
<td>1206.28</td>
<td></td>
</tr>
</tbody>
</table>

\( %\Delta \) = Ratio score of the primary outcome measure; GMD = Gray matter density (ml/voxel); M = mean, SCD = Scalp-to-cortex distance (mm), SD = Standard deviation.

\(^1\) Baas et al. 2014
\(^2\) Koops et al. 2016
\(^3\) Slotema et al. 2011
\(^4\) De Weijer et al. 2014
\(^5\) Dlabac-de Lange et al. 2015
Figure 2.
Boxplot of the ratio scores (delta) representing treatment efficacy, presented per study.

Table 5. Non-parametric correlation coefficients for associations between morphological measures and treatment efficacy. The morphological measures are presented for the total group (total), separately for the four AVH treatment trials (AVH), three AVH treatment trials using the 10-20 EEG method for localization (AVH_10_20), and negative symptom treatment trial (neg).

<table>
<thead>
<tr>
<th>Morphological Measure</th>
<th>Kendall’s Tau with %Δ</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD_total</td>
<td>.1</td>
<td>.14</td>
</tr>
<tr>
<td>SCD_AVH</td>
<td>.17</td>
<td>.05*</td>
</tr>
<tr>
<td>SCD_AVH_10_20</td>
<td>.22</td>
<td>.02*</td>
</tr>
<tr>
<td>SCD_neg</td>
<td>-.15</td>
<td>.23</td>
</tr>
<tr>
<td>GMD_total</td>
<td>.11</td>
<td>.1</td>
</tr>
<tr>
<td>GMD_AVH</td>
<td>.12</td>
<td>.11</td>
</tr>
<tr>
<td>GMD_AVH_10_20</td>
<td>.17</td>
<td>.05*</td>
</tr>
<tr>
<td>GMD_neg</td>
<td>-.06</td>
<td>39</td>
</tr>
<tr>
<td>TBV_total</td>
<td>.03</td>
<td>.37</td>
</tr>
<tr>
<td>TBV_AVH</td>
<td>.06</td>
<td>.26</td>
</tr>
<tr>
<td>TBV_AVH_10_20</td>
<td>.05</td>
<td>.33</td>
</tr>
<tr>
<td>TBV_neg</td>
<td>-.14</td>
<td>.24</td>
</tr>
</tbody>
</table>

GMD = Gray matter density; SCD = Scalp-to-cortex distance; TBV = Total brain volume.
*p < .05 (one-sided)
Primary motor cortex

Data on the motor threshold were available for 54 patients in total. The stepwise linear regression models demonstrated that the motor threshold could not be predicted by SCD or GMD of the primary motor cortex. SCD of the stimulated region correlated significantly with SCD of the primary motor cortex ($r = .31, p = .001$). This was also the case for GMD of both regions ($r = .36, p<0.001$). Furthermore, SCD of the stimulated region was significantly shorter ($t(67) = -6.86, p<0.001$) than SCD of the motor cortex. GMD of the stimulated region was significantly higher than GMD of the motor cortex ($t(67) = 8.38, p<0.001$).

Discussion

The primary aim of the present study was to investigate possible morphological predictors of rTMS treatment efficacy. For this purpose, data from multiple clinical trials aimed at alleviation of AVH and negative symptoms in patients with schizophrenia were combined. All AVH trials used inhibitory rTMS to reduce neural activity within the temporo-parietal region, while the negative symptom trial targeted the DLPFC with excitatory rTMS. First, it was evaluated whether morphological predictors (specifically scalp-to-cortex distance and gray matter density) could be established for rTMS treatment efficacy in general, therefore data from all treatment trials was combined in that analysis. Subsequently, it was investigated whether the predictive value of the morphological variables could be dependent on location of stimulation (in relation to targeted symptomatology). Within these follow-up analyses, associations between treatment efficacy and morphological characteristics were separately tested for patients that received rTMS over different locations (the DLPFC and the TPJ, also excluding fMRI-guided rTMS because stimulation was provided on other temporal or parietal regions). Our results demonstrate that a shorter distance between the point of stimulation at the scalp and the underlying temporo-parietal cortex was associated with greater treatment efficacy in patients treated for AVH. This relationship became stronger when the participants that received fMRI-guided rTMS were left out from the analyses. No such relationship was found for treatment efficacy in trials aimed at improving negative symptoms. Furthermore, the density of the stimulated gray matter was associated with treatment efficacy in patients treated for AVH, but not for negative symptoms. Our results are in accordance with a previous study on morphological predictors in rTMS treatment efficacy for AVH in patients with schizophrenia (Nathou et al., 2015), in which shorter scalp-to-cortex distance (SCD) and greater gray matter density (GMD) were reported to predict treatment efficacy.

In the rTMS trials for AVH that were included in present study, the majority of the participants received stimulation of the temporo-parietal region. A large body of evidence has shown smaller gray matter volumes of this region in patients with schizophrenia (Chan et al., 2009), and particularly of this region in association with positive symptom severity including AVH (Honea, Crow, Passingham, & Mackay, 2005; Modinos et al., 2013). For studies investigating structural differences in relation to negative symptoms, results are more inconclusive and do not always indicate the DLPFC as an area of reduced gray matter volume (Honea et al., 2005; Koutsouleris et al., 2008; Ozdemir et al., 2012; T. Zhang, Koutsouleris, Meisenzahl, & Davatzikos, 2015). Reduced gray matter volumes of the temporo-parietal region in patients with schizophrenia may supposedly result in a larger area between the scalp and the cortex filled with cerebrospinal fluid (CSF). Due to the fact that the conductivity of CSF is much greater than that of brain tissue, the induced electrical current may spread more easily if there is more CSF, therefore losing its focality and strength. Consequently, the chance
to reach the underlying cortex will decrease (Bijsterbosch, Barker, Lee, & Woodruff, 2012). Overall, these findings might explain why SCD was predictive of treatment efficacy in AVH trials, while it was not in the negative symptom trial. Furthermore, the predictive value of SCD on AVH improvement increased after only including subjects that were stimulated on the TPJ as determined with the international 10-20 system. It may be that the coordinates as determined with fMRI were generally not as superficially located as the 10-20 EEG location. As the effective depth of stimulation may not be more than 1-2 cm into the cortex (Roth, Flashman, & McAllister, 2007), the magnetic pulses may have lost much of their strength before reaching the fMRI-guided target areas.

As a result of previously reported gray matter reductions in patients with schizophrenia (Chan et al., 2009), the thickness of the cortical layer and its density may also be reduced. Both cortical thickness and density have been associated with cortical excitability in healthy subjects (Conde et al., 2012; List et al., 2013). Conceivably, reductions in gray matter may negatively influence its excitability and the efficacy of rTMS. Indeed, a significant positive correlation between GMD and treatment efficacy in the sample of patients that received rTMS on the TPJ (EEG location) demonstrates the importance of GMD. However, this association was not present in the AVH sample when the fMRI-guided participants were included, nor in the total sample. Hence, our findings on GMD were less pronounced than the findings reported by Nathou et al. (2015). This could possibly be ascribed to differences in methodologies: Nathou et al. (2015) measured GMD of the superior temporal sulcus, which was located less superficial than the region we measured (directly underneath the coil). We chose superficial regions for GMD calculation as it can be assumed that the superficial gray matter may benefit most from rTMS effects. Uniformity in the applied methodology may increase the chance of finding similar results on GMD. In addition, there were differences in stimulation parameters, duration, and differences in efficacy of the applied treatment protocols compared to Nathou et al. (2015). However, we can only speculate if these differences contribute to the lack of associations that we found.

In addition to the SCD and GMD measurements of the stimulated regions, these morphological measures were also investigated for the primary motor cortex. Interestingly, although GMD and SCD of the stimulated region and the primary motor cortex were positively correlated, GMD of the stimulated region was significantly higher and SCD was significantly lower compared to the motor region. This is in line with a study by Stokes et al. (2005), in which scalp-to-cortex distances were compared between several brain regions that are frequent targets for rTMS. The authors reported larger distances for the motor cortex than for the superior temporal gyrus and middle temporal gyrus (Stokes et al., 2005). It is thus conceivable that the stimulation intensity for the TPJ area as determined with the motor threshold procedure is sufficiently high.

One of the key strengths of this study was that the total number of included patients was relatively high, as compared to the study by Nathou et al. (2015) (who included 15 subjects). Although these patients were combined from multiple treatment trials, treatment efficacy did not significantly differ amongst the included studies, the data from the different trials were analyzed using a uniform procedure, and analyses were repeated for trials targeting different anatomical locations (trials targeting the TPJ and DLPFC), and using different localization methods (the international 10/20 system versus individually fMRI guided rTMS). However, a note of caution is in place since we combined trials
that used various methodologies of neurostimulation. Studies were not consistent in the applied rTMS paradigm nor in the duration of treatment. Due to small sample sizes of the individual AVH studies, follow-up analyses in this context were not performed. Furthermore, the group size in the negative symptom trial was small and lack of power may be causative for the absence of a predictive value of SCD and GMD in this group. Hence, the role of possible morphological predictors for rTMS treatment efficacy for negative symptoms requires further investigation in larger samples and preferably in more homogeneous samples with respect to rTMS treatment protocols. Furthermore, it would be of interest for future research to investigate other possible morphological predictors that could be of influence on the efficacy of neurostimulation, including baseline patterns of cerebral blood flow (for review see Silverstein et al., 2015; Weiduschat & Dubin, 2013), and the level of activation of the underlying brain networks during stimulation (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011; Antal, Terney, Poreisz, & Paulus, 2007; Fregni et al., 2006; Kimbrell et al., 1999; Kujirai, Kujirai, Sinkjaer, & Rothwell, 2006; Micoulaud-Franchi et al., 2013).

To conclude, SCD and GMD of the TPJ are associated with rTMS treatment efficacy in trials aimed to reduce severe AVH, even though treatment efficacy in the included samples was relatively low. Our data suggests that rTMS protocols need to be individually adjusted or new treatment protocols need to be developed in order to assure that the rTMS pulses reach the cortex in sufficient strength and number. Furthermore, if future studies confirm the predictive value of SCD for efficacy of rTMS over the temporo-parietal region and find additional associations with other morphological, demographical, and clinical predictors, this will clearly be of clinical significance as it can aid approaches that are individually tailored to each patient.

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