Open access • Journal Article • DOI:10.3109/15622975.2013.872295

Accelerated HF-rTMS in treatment-resistant unipolar depression: Insights from subgenual anterior cingulate functional connectivity — Source link

Chris Baeken, Daniele Marinazzo, Guo-Rong Wu, Peter Van Schuerbeek ...+6 more authors

Institutions: Ghent University, University of Electronic Science and Technology of China

Published on: 12 May 2014 - World Journal of Biological Psychiatry (World J Biol Psychiatry)

Topics: Dorsolateral prefrontal cortex, Prefrontal cortex and Anterior cingulate cortex

Accepted for publication in "World Journal of Biological Psychiatry"

Note: This is an uncorrected version of an author's manuscript accepted for publication. Copyediting, typesetting, and review of the resulting proofs will be undertaken on this manuscript before final publication. During production and pre-press, errors may be discovered that could affect the content.

Accelerated HF-rTMS in treatment-resistant unipolar depression: insights from subgenual anterior cingulate functional connectivity

Chris Baeken[1,2,3,CA], Daniele Marinazzo[4], Guo-Rong Wu[4,5], Peter Van Schuerbeek[6], Johan De Mey[6], Igor Marchetti[7], Marie-Anne Vanderhasselt[7], Jonathan Remue[7], Robert Luypaert[6], Rudi De Raedt[7]

[1] Department of Psychiatry and Medical Psychology, Ghent University, Ghent, Belgium

[2] Department of Psychiatry, University Hospital (UZBrussel), Brussels, Belgium

[3] Ghent Experimental Psychiatry (GHEP) lab, Ghent University, Ghent, Belgium

[4] Department of Data Analysis, Ghent University, Ghent, Belgium.

[5] Key Laboratory for NeuroInformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, China.

[6] Department of Radiology and Medical Imaging, University Hospital (UZBrussel), Brussels, Belgium

[7] Department of Experimental Clinical and Health Psychology, Ghent University, Ghent, Belgium

---

[CA] Corresponding author: Chris Baeken, M.D, Ph.D. Department of Psychiatry and Medical Psychology Ghent University, De Pintelaan 185 – 9000 Ghent, Belgium. Tel: +32 (0)9 332 5543. Fax: +32 (0)9 332 4989. E-mail: chris.baeken@UGent.be

Abstract

Objectives: Intensified repetitive transcranial magnetic stimulation (rTMS) applied to the left dorsolateral prefrontal cortex (DLPFC) may result in fast clinical responses in treatment resistant depression (TRD). In these kinds of patients, subgenual anterior cingulate cortex (sgACC) functional connectivity (FC) seems to be consistently disturbed. So far, no de novo data on the relationship between sgACC FC changes and clinical efficacy of accelerated rTMS were available.

Methods: Twenty unipolar TRD patients, all at least stage III treatment resistant, were recruited in a randomized sham-controlled crossover high-frequency (HF)-rTMS treatment study. Resting-state (rs) functional MRI scans were collected at baseline and at the end of treatment.

Results: HF-rTMS responders showed significantly stronger resting-state functional connectivity (rsFC) anti-correlation between the sgACC and parts of the left superior medial prefrontal cortex. After successful treatment an inverted relative strength of the anti-correlations was observed in the perigenual prefrontal cortex (pgPFC). No effects on sgACC rsFC were observed in non-responders.

Conclusions: Strong rsFC anti-correlation between the sgACC and parts of the left prefrontal cortex could be indicative of a beneficial outcome. Accelerated HF-rTMS treatment designs have the potential to acutely adjust deregulated sgACC neuronal networks in TRD patients.

Key words: HF-rTMS, major depression, treatment-resistance, subgenual anterior cingulate cortex, functional connectivity

1. Introduction

Major depression is a severe mental health problem affecting millions worldwide (Nemeroff 2007a). Unfortunately, many depressed patients do not respond to the available pharmacological treatment. This is referred to as treatment-resistant depression (TRD) (Fava 2003; Nemeroff 2007b). When challenged with clinical non-response, treatment options are limited (Shelton et al. 2010; Ward and Irazoqui 2010). In the last two decades, repetitive transcranial magnetic stimulation (rTMS) has been used to treat depressed patients, including those who do not benefit from the pharmacological approaches (Padberg and George 2009; George and Post 2011). Classically, the left dorsolateral prefrontal cortex (DLPFC) is targeted with high-frequency (HF) rTMS, a protocol found to frequently result in beneficial outcomes (Schutter 2009; Fitzgerald and Daskalakis 2012). Of note, other neurostimulation techniques, such as transcranial direct current stimulation (tDCS), have also shown beneficial effects in the treatment of depression (Brunoni et al., 2013). The rationale for using (predominantly) the left DLPFC as the rTMS target area originates from brain-imaging research, which has indicated that unipolar depressed patients show decreased neuronal activity in this prefrontal region (Mayberg 2003; George et al. 2003; Drevets et al. 2008a). After rTMS application, neuronal changes are not only observed in the area under the stimulation coil, but also in areas synaptically connected to the targeted region, ipsi- as well as contralateral (Paus et al. 2001; Paus and Barrett 2004). These neuronal rTMS effects are found both in healthy subjects and patients suffering from major depression (Knoch et al. 2006; Baeken et al. 2009; Kito et al. 2012).

In contrast to hypo-activities in the DLPFC and rostral anterior cingulate cortical (rACC) areas, increased metabolic activity in the ventromedial prefrontal cortex (vmPFC) is often observed in the depressive state (Price and Drevets 2012). The subgenual anterior

cingulate cortex (sgACC: Brodmann area (BA) 25) has consistently been shown to be metabolically hyperactive during (treatment resistant) depressive episodes (Drevets et al. 1997, 2008b; Mayberg et al. 2005). The sgACC is part of distributed corticolimbic neurocircuits implicated in 'visceromotor' functions and in modulating affect, such as sadness and ruminative thought patterns (Disner et al. 2011; Smith et al. 2011; Davey et al. 2012a). In general, successful pharmacotherapy attenuates this sgACC metabolic hyperactivity (Mayberg 2003). Additionally, non-pharmacological strategies for the treatment of refractory depression, such as deep brain stimulation (DBS) and anterior cingulotomy, specifically target the sgACC with the intention to interrupt this overactive limbic region (Dougherty et al. 2003; Mayberg et al. 2005; Johansen-Berg et al. 2008; Steele et al. 2008). The clinical efficacy of nervus vagus stimulation (VNS) therapy, electroconvulsive therapy (ECT) and rTMS in TRD seems to correlate with decreases in sgACC activity (Nobler et al. 2001; Mottaghy et al. 2002; Zobel et al. 2005). Accordingly, it has been proposed that sgACC hyperactivity could be a potential neurobiological marker for TRD (Greicius et al. 2007; Guinjoan et al. 2010; Holtzheimer and Mayberg 2011).

From a clinical neuroscience perspective, the combination of rTMS treatment algorithms with brain imaging techniques holds potential for elucidating the neurobiological effects of neurostimulation on the human 'depressed brain'. Major depression can be conceptualized as a neural-network-level disease, involving functional connectivity deregulation in the prefrontal and limbic areas (Sheline et al. 2010; Carballedo et al. 2011). Recent research (Fox and Greicius 2010; Veer et al. 2010; Marchetti et al. 2012; Fox et al. 2012 a, b; Cisler et al. 2013) has focused on this deregulated FC. The combination of rTMS and functional connectivity MRI (fcMRI) may significantly contribute to the diagnosis and treatment of psychiatric illnesses with

deregulated network pathologies such as major depression (Fox et al. 2012 b). Moreover, the combination of these techniques may increase our neurobiological understanding of clinical response and the phenomenon of non-response, potentially accelerating the translation of both techniques into the clinical realm. Fox et al. (2012 a), for instance, have evaluated the resting state FC (rsFC) of different regions within the left DLPFC with the sgACC as seed, in view of the selection of optimal rTMS target sites. They showed that target sites with the best rTMS antidepressant efficacy exhibited the strongest anti-correlated rsFC in the sgACC. These sites were localized in the more anterior parts of the left DLPFC. One aspect of this important work was the use of existing information on the responsiveness of the depressed subjects (no de novo information acquisition as part of the experimental set-up).

Another avenue for improving treatment effects in depressed patients, has involved the use of more potent rTMS stimulation (e.g. suprathreshold sessions with the delivery of a higher number of pulses) and of neuronavigation techniques for identifying the DLPFC target (Gershon et al. 2003; Fitzgerald et al. 2009). In an effort to acutely affect mood in TRD patients, recent research designs have further intensified treatment by delivering the HF-rTMS sessions in a few days instead of spreading them over several weeks (Holtzheimer et al. 2010; Hadley et al. 2011; Zeeuws et al. 2011).

Although a number of fcMRI studies have focused on non-refractory major depression (for an overview, see Wang et al. 2012), no studies have so far investigated the influence of intensified HF-rTMS treatment on FC in TRD. Also, combined de novo data on the effect of rTMS on FC and clinical efficacy have so far been lacking. Consequently, in this sham-controlled HF-rTMS study of a group of at least stage III unipolar TRD patients, we investigate in the impact of accelerated rTMS treatment on functional connectivity and depression status. In view of the well documented sgACC

abnormalities in major depression, we focused on this region as seed area for the fcMRI. Assuming the clinical effects of HF-rTMS to be related to the existence of rsFC anti-correlation between the stimulated area and the sgACC (Fox et al. 2012a), we expected that, at baseline, responders would display stronger anti-correlation patterns between sgACC and the prefrontal cortical areas than non-responders. In addition, we hypothesized that in responders the anti-correlation between the sgACC and the left prefrontal cortex would decrease after treatment, while we did not anticipate comparable FC changes in non-responders.

2. Materials and Methods

This study was part of a larger project investigating the influence of HF-rTMS on various neurocognitive markers. It was approved by the local ethics committee of the UZBrussel and all subjects gave written informed consent.

2.1 Subjects

The study group consisted of twenty antidepressant-free, unipolar severely depressed patients (HDRS: 25.65 ± 6.13; age: 48.80 years ± 12.76; 13 females), selected using the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). All were right-handed (Van Strien and Van Beek 2000) and at least stage III treatment- resistant, as described by Rush et al. (2003): they had received a minimum of two unsuccessful treatment trials with serotonin reuptake inhibitors/ noradrenaline and/or serotonin reuptake inhibitors (SSRI/NSRI) and one failed clinical trial with a tricyclic antidepressant (TCA). Exclusion criteria were a current or past history of epilepsy, neurosurgical interventions, having a pacemaker, metallic or magnetic objects in the brain, alcohol dependence and any suicide attempts within 6 months before the start of the study. Because concomitant antidepressant treatment can confound outcome results, all patients went through a medication washout period before entering the study supervised by their physician, guaranteeing that all TRD patients were free from any antidepressant (AD), neuroleptic and mood stabilizer. All TRD patients were at least two weeks free from these agents (three weeks when on fluoxetine) before entering the HF-rTMS treatment protocol. Only habitual benzodiazepine agents were allowed. The maximum allowed dose of benzodiazepines was the equivalent of 20 mg diazepam. These benzodiazepines equivalent doses are described by the British National Formulary (No. 64. London: British Medical Association and Royal Pharmaceutical Society of Great Britain; September 2012, p212-220). During this psychotropic-free two week period,

besides steady doses of benzodiazepine, no withdrawal symptoms were recorded. Any psychopharmacological changes during the protocol were considered valid reasons for exclusion from the study.

2.2 Protocol

We used a sham-controlled, cross-over experimental design covering two weeks (Figure 1). Half of the patients were selected at random to receive in the first week real HF-rTMS delivered to the left DLPFC, the other half undergoing sham treatment during that week. These roles were reversed in the second week. At the start of the first week (time T_0), the depression severity of each subject was assessed by a certified psychiatrist unrelated to the actual HF-rTMS treatment of the patient, using the 17-item Hamilton Depression Rating Scale (HDRS; Hamilton 1967). The patients were re-assessed in the same way at the end of this week (T_1) and at the end of the second week (T_2) of the stimulation protocol. At times T_0, T_1 and T_2, the patients underwent fcMRI.

2.3 HF-rTMS Stimulation

The treatment protocol of 20 HF-rTMS sessions was spread over 4 days of each week, yielding a total of 31200 stimuli. See also Figure 1. On each stimulation day, usually in the afternoon between 2:00 pm and 6:00 pm, the patient received 5 sessions with an intersession delay of 15 to 20 minutes. Patients were kept unaware of the type of stimulation; they wore earplugs and were blindfolded. For the application of HF-rTMS we used a Super Rapid Magstim high-speed magnetic stimulator (Magstim Company Limited, Wales, UK), connected to a 70 mm figure-of-eight-shaped coil. Before each

session, the resting motor threshold (RMT) of each individual was determined using electromyography. Single pulse TMS in combination with motor evoked potentials (MEP), measured with surface electromyography (EMG) registration determined the optimal muscular response (right abductor pollicis brevis muscle). We adjusted stimulus intensity until positive MEP responses were recorded and clear thumb muscular abduction was observed. Before the selected part of the cortex was accepted as the motor cortex related to the contralateral abductor pollicis brevis (APB) muscle, positive MEP responses of at least 50 μV (peak-to-peak amplitude) had to be produced in at least five out of ten consecutive trials. A stimulation intensity of 110 % of the subject's RMT of the right APB muscle was used. In order to accurately target the left DLPFC, the precise stimulation site was determined using three-dimensional Magnetic Resonance Imaging (3D-MRI) (see also Peleman et al. 2010). In the sham condition, the coil was held at an angle of 90°, only resting on the scalp with one lateral edge. In each high-frequency (20Hz) stimulation session, subjects received forty trains of 1.9 seconds duration, separated by an intertrain interval of 12 seconds (1560 pulses per session). The rTMS parameters were in each separate HF-rTMS session are in agreement with current safety guidelines (Wassermann et al. 1998; Rossi et al. 2009).

2.4 Brain imaging

To obtain individual anatomical information, all subjects underwent a T1-weighted MRI (3D-TFE,voxel size 1 1 1 mm) of the brain using a 3T Achieva MR scanner (Philips, Best, The Netherlands). All post processing was done on a ViewForum console (Philips). We located the left DLPFC visually on the 3D surface rendering of the brain based on the known gyral morphology and marked the centre part of the midprefrontal gyrus as the the left DLPFC target (Brodmann 9/46; MNI coordinates: x= −45, y= 30, z= 31). The corresponding coil position was found by determining the perpendicular projection of this point on the scalp (see also Peleman et al., 2010 for an extensive description).

During the resting-state measurements, which involved exactly five minutes of scanning, the participants were asked to stay awake with their eyes closed. To reduce sensory confounds as much as possible, the light in the room was dimmed during scanning. After the scan, we certified that they had been awake throughout the scan and had complied with the instructions. All resting-state fMRI scans were performed on a Monday afternoon between 3:00 pm and 6:00 pm.

The scans were performed on a 3T Philips Achieva MRI system (Philips, Best, The Netherlands) with an eight channel SENSE head coil. The fMRI measurement was done using a SE-EPI sequence (TR/TE=3000/70ms; flip angle=90°; FOV=230x230mm²; resolution=1.80x1.80mm²; slice thickness/gap=4.00/1.00mm; number of slices=24; number of dynamics=100; time resolution=3000ms). After the fMRI scan, a 3D anatomical scan was taken for use as anatomical underlay for the results using a 3D T1 TFE sequence (TR/TE=12.00/3.71; flip angle=10°; FOV=240x240x200mm³; resolution=1.00x1.00x2.00mm³; number of slices=100).

2.5 Data analysis

2.5.1 Behavioral data

All behavioral data were analyzed using SPSS 19 (IBM, Statistical Package for the Social Sciences, Chicago). Clinical response was defined as a 50% reduction of the baseline HDRS score after each treatment condition for one week. See also Table 1 and Figure 1. To evaluate the differences between responders and non-responders, we applied independent T or X^2 tests where appropriate. The significance level was set at $p< 0.05$ for all analyses.

2.5.2 Imaging data

The fMRI data were analyzed using the SPM8 software (Wellcome Department of Cognitive Neurology, London, UK). The images were realigned to the first volume to correct for head movements. After the realignment step, a slice-time correction was performed to correct for small differences in the time-offset of consecutively measured slices. Subsequently, all brain volumes were normalized to the EPI MNI template, then resampled to 3-mm isotropic voxels. The anatomical scans were normalized to the T1 MNI template.

Several additional processing steps were preceding the analysis of the voxel-based correlations. The data were linearly detrended and band-pass filtered (0.01-0.08Hz). Several spurious or nonspecific sources of variance were removed from the data through linear regression: 1) the six head-motion parameters obtained in the realignment step, 2) the signal from a region in the cerebrospinal fluid, 3) the signal from a region centered in the white matter, 4) the whole-brain signal. Correlation maps were then obtained by

extracting the BOLD time course from the seed region and computing the correlation coefficients between that time course and the time courses in all other brain voxels. In our case the seed region was a 6-mm-diameter sphere centered on a point with MNI coordinates (x= 1, y= 25, z= -11), designed to encompass the sgACC. These MNI coordinates were selected following the recent paper of Cisler et al. (2013), defining centroids of nodes comprising an affective cognitive network. To combine results across subjects and compute statistical significance, Fisher's r-to-Z transformation was used to convert these correlation maps into Z-maps characterizing the rsFC of the seed region in each point.

These rsFC maps were submitted in SPM8 to a random-effects two-way ANOVA, containing Age as covariate, Response (positive, negative) as between-subject factor and Time (baseline, post-treatment) as within-subject factor. Because at T_1 only two patients could be identified as clinical responders, the ANOVA with the rsFC maps of the scan at T_1 could not be performed. Hence, Response was defined as a 50% reduction of the baseline HDRS score at T_0 compared to the HDRS score at the end of the entire experimental protocol at T_2. The *F*-test for investigating the presence of an interaction effect between Response at T_2 and Time was thresholded using the Alphasim correction as implemented in the SPM REST toolbox (restfmri.net/forum/) at $p < 0.05$ (cluster size: 389 voxels). Two-sample *T*-tests were performed post hoc to further investigate the characteristics of the interaction, comparing responders and non-responders. These analyses were constrained to the region of significant Response x Time interaction, thresholded at a cluster extent (K) of 25 voxels and a voxel significance level of 0.05 according to the Alphasim correction.

3. Results

3.1 Behavioral results

The results of the behavioral data analysis are summarized in Table 1. Ten subjects received real HF-rTMS treatment in the first week and sham in the second week; 10 followed the reverse order. A total of seven subjects (35% of the total) were identified as responders at the end of the two-week study protocol. All responders were identified after real HF-rTMS. However, the two responders that were identified at the end of the first week remained responders after their sham sessions in week two. Five subjects responded in the second week after receiving their real HF-rTMS treatment during that week, after not having responded to sham treatment in their first week (for the full outcome results, see Baeken et al., *in press*). Of note, the participants in the current study are not exactly the same as no rsFMRI data could be collected from one patient with a nervus vagus stimulator (VNS) implanted for a previous depressive episode. Baseline HDRS measurements were not significantly different between responders and non-responders ($t(18)=0.51$, $p=0.79$ at T_0).

On the demographics side, we noted no difference between responders and non-responders in gender ($X^2(1)=0.20$, $p=0.66$), but a marginal difference in age ($t(18)=2.00$, $p=0.06$).

3.2 Functional connectivity results

These results are summarized in Table 2 and Figures 2 and 3. Due to the unavailability of the scanner at T2 only 12 second scans (fcMRI end) could be performed (7 non-responders and 5 responders). Although before entering the HF-rTMS treatment protocol the baseline scan could be programmed for every TRD patient, because our MRI scan is mainly used for clinical purposes, it was not always possible to perform the T_2 scan within the set time period of two weeks. Because we did not want to perform this fMRI three or four weeks after the initiation of the treatment protocol, introducing too much variability into our rsFC data, these post-treatment fMRI scans were not performed. The two-way ANOVA yielded a significant Response x Time interaction cluster located in the superior medial frontal gyrus (k= 435; MNI coordinates: x= 0, y= 60, z= 21; see Figure 2). The post hoc analysis restricted to this region showed that, at baseline, the (anti-correlated) sgACC rsFC was significantly stronger in responders than in non-responders in two clusters: the central part of the medial superior frontal gyrus (BA 10; k= 108; MNI coordinates: x= 0, y= 48, z= 30) and the left superior frontal gyrus (BA 10; k= 47; MNI coordinates: x= -24, y= 51, z= 27). The post-hoc analysis also showed that, after the HF-rTMS treatment, compared to non-responders the responders displayed significantly stronger sgACC rsFC correlation between the perigenual anterior cingulate (pgACC)/superior medial frontal gyrus (BA 32/10; k= 298; MNI coordinates: x= 6, y= 45, z= 9). These midprefrontal areas are part of the perigenual prefrontal cortex (pgPFC) (Price and Drevets, 2012).

In particular to verify whether HF-rTMS treatment did affect sgACC rsFC in clinical non-responders, we extracted the individual time courses out Response x Time interaction cluster in MarsBaR (Brett *et al.* 2002) for responders and non-responders, before and after treatment, age corrected.

For the rsFC based on these time courses a two-way ANOVA, Response (positive, negative) as between-subject factor and Time (baseline, post-treatment) as within-subject factor showed a significant main effect of Time (F (1,10)= 11.25, p<.01) but not for Response (F (1,10)= 0.1, p=.92). However, the interaction effect between Response and Time was highly significant (F (1,10)= 36.86, p<.01). See also Figure 4. Wilcoxon paired T-tests confirmed that successful HF-rTMS treatment resulted in an inverse correlation effect between sgACC rsFC and the superior medial frontal gyrus interaction cluster (mean baseline = -1.0 (.15), mean after HF-rTMS= .14 (.12): z= -2.02, N (ties)=5, p= .04). No significant changes were observed for non-responders (mean baseline = -0.03 (.07), mean after HF-rTMS= -.03 (.02): z= -1.69, N (ties)=7, p= .09).

4. Discussion

To our knowledge, this is the first study in which the effect of accelerated HF-rTMS on functional connectivity and de novo measured depression status was evaluated. A sample of at least stage III treatment-resistant depressed patients was investigated. The rTMS targeted the center of the left DLPFC (BA 46/9) under MRI guidance. At the behavioral level (depression severity symptoms as measured by the HDRS) we could not discriminate responders from non-responders at baseline, but at the end of the treatment period the group of responders was distinguished from the non-responders by a significantly lower HDRS score**.** See Figure 4 (left). Our rsFC results corroborated the importance of the sgACC as key structure involved in clinical response to rTMS in TRD. At baseline, the HF-rTMS responders showed stronger anti-correlated sgACC rsFC than non-responders in parts of the prefrontal cortex (BA 10), predominantly on the left. After treatment, a reversal of the relative strengths of the anti-correlated rsFc in responders vs non-responders was found, covering parts of the pgPFC (BA 32/10). See Figure 4 (right).

Our rsFC results support the recent observations of Fox and colleagues (2012 a), in which a stronger anti-correlation at baseline between the sgACC and specific stimulation sites in the (dorsolateral) prefrontal cortex was reported to have a more beneficial clinical outcome for rTMS. In line with this reasoning one could speculate that only TRD patients displaying such anti-correlation pattern may be susceptible to these intensified HF-rTMS treatment algorithms, as HF-rTMS specifically influences this anti-correlation. This is confirmed here (Figure 4 (right)): at baseline the responsive subjects in our sample exhibit strong anti-correlation, while the non-responsive ones on average show a slightly positive correlation. After successful treatment, the roles are inverted: the anti-correlation has become slightly positive in responders, while in non-responders the correlation has become slightly negative, although not significant.

Because the interpretation of anti-correlations between functional networks has not been clarified yet (Fox and Greicius 2010; Fox et al. 2012 b), at this point one can speculate that this observed anti-correlation reflects a consistent neurobiological datum in major depression: that is hyperactivity in the sgACC and hypo-activity in the left (dorsolateral and medial) prefrontal cortical areas (Mayberg 2003; Drevets et al. 2008a). Of course this assumption is based on other neuroimaging techniques not necessarily measuring the same neuronal processes. Nevertheless, the stronger FC observed after successful HF-rTMS treatment between the sgACC and parts of the left superior frontal gyrus (BA 10) suggests a reversal of neuronal activity within these networks. This reversal pattern has also been reported after successful psychopharmacotherapy and neurostimulation techniques such as DBS and rTMS (Mayberg et al. 2005; Drevets et al. 2008 b; Baeken et al. 2009). These brain imaging data could indicate that this reversal pattern is essential for depression improvement, regardless of the used intervention. However, this was measured with different imaging techniques making it difficult for direct comparison. Further, our data do not imply that HF-rTMS non-responders display no similar anti-correlation pattern, only that HF-rTMS responders have stronger FC anti-correlations between these two areas at baseline. Nevertheless, a reversal of FC between sgACC and similar parts of the PFC could be essential for clinical response and remission in depressed patients, explaining to some extent the lack of significant FC changes between these areas in HF-rTMS non-responders. Whether this anti-correlation is due to the combination of specific neuronal dysfunctions between the two regions or there is a compensatory effect of one area over the other remains to be clarified (Fox et al. 2012 b). Albeit we targeted the center of the left DLPFC (BA 46/9), we did not observe the anti-correlation within this area and the sgACC suggested by Fox et al. (2012 a). However, in our TRD sample we did find a significant anti-correlation pattern between the sgACC and

parts of the left superior medial frontal gyrus (BA 10), anterior to our stimulation target area. Our current results imply that to effectively treat TRD patients even more anterior located stimulation areas could be targeted. Whereas Fox et al. (2012 a) evaluated classical rTMS paradigms in healthy as well as in depressed patients, we used a novel intensified HF-rTMS paradigm in a sample of TRD patients, which makes it difficult to compare. Furthermore, although we corrected for age in our FC analyses, HF-rTMS responders were globally younger than non-responders. On the one hand, it has been documented that age differences may influence anti-correlation analyses (Koch et al. 2010; Wu et al. 2011). The observed stronger anti-correlation between the sgACC and parts of the (left) prefrontal cortex in responders may to some extent explain as to why relatively younger depressed patients could benefit from this form of treatment.

Our whole brain analyses revealed that the FC between the sgACC and parts in the pgPFC became stronger in clinical HF-rTMS responders. Ventral-rostral portions of the ACC and parts of the vmPFC have a regulatory role with respect to limbic regions involved in generating emotional responses (Etkin et al. 2011). The pgPFC is implicated in mechanisms of consciousness and emotional awareness (Amting et al. 2010). Given the role of the pgPFC in emotion-regulative processes (Ochsner and Gross 2005) the observed enhanced functional connectivity between sgACC and pgPFC could be essential for the suppression of depressive symptoms. In addition, it has been reported that AD responders show higher pgACC metabolic states and electrophysiological activity than AD nonresponsive patients (Mayberg et al. 1997; Pizzagalli et al. 2001). Of note, in an open ^{18}FDG-PET study examining a different but similar sample of at least stage III TRD patients and stimulating exactly the same anatomical coordinates within the left DLPFC, we found that a positive clinical response was associated with increases in both (dorsolateral) prefrontal and pgACC (BA 32) metabolic activities (Baeken et al. 2009).

Similar pgACC increases in neuronal activity after effective add-on left-sided HF-rTMS treatments were reported in TRD patients (Kito et al. 2008, 2012). Of interest, in contrast to the sgACC, these ventral parts of the ACC are synaptically interconnected with the DLPFC (BA 9/46) (Paus and Barrrett, 2004) and the increased FC in the pgACC could be indicative for the improved cognitive regulation of affect (Davey et al., 2012 b). Further, we found that successful stimulation resulted in stronger FC between the sgACC seed and parts of the left superior frontal gyrus (BA 10). Being part of a resting state or default mode network (DMN), these areas are activated when individuals make self-relevant affective decisions (Wendelken et al. 2008; Andrews-Hanna et al. 2010). These rostral parts of the medial prefrontal cortex are associated with emotion regulation, sustained attention, memory, and mentalizing processes (Ramnani and Owen 2004; Amodio and Frith 2006; Burgess et al. 2007a, b). Left and not right BA 10 has been related to adaptive changes of attentional resource allocation, in the absence of awareness and learned contingencies (Pollmann and Manginelli 2009). Altogether, the stronger FC between the sgACC and the two areas in the pgPFC, related to clinical response, could imply that firstly, the pgACC area regains better emotional control over the unrestricted arousing responses found in the overactive sgACC during depressive episodes; secondly, there is improvement on the cognitive level, influencing attention, memory and empathy processes. The lack of FC change during non-response is in line with the paper of Hamilton and colleagues (2011) where continuous activities in the ventral parts of the ACC and the medial prefrontal cortex were mutually reinforcing major depressive symptoms.

Besides the relatively small sample size, some limitations have to be discussed. As no long-term effects were examined, the interpretations of this study design are restricted to the immediate effects on FC, specifically for TRD patients. In spite of our clear a priori

hypothesis on sgACC involvement in TRD, other key regions in major depression, such as the orbital frontal cortex, hippocampus, insula and amygdala are often found to be deregulated (Price and Drevets 2010, 2012), but these were not examined here. Another major limitation of the study is that we did not include the analyses of an intermediate fMRI scan into our analyses because there were not enough responders as measured just after the first week. Without the analyses of the intermediate scan the interpretation of the current results remains limited to the effects of accelerated HF-rTMS of a treatment protocol which includes both real and sham stimulation. However, all patients who did not respond after real HF-rTMS in the first week also remained clinical non-responder after sham week two, implying the inefficacy of sham (see Baeken et al., *in press*). Further, based on the meta-analysis of Berlim and colleagues (2013) concerning blinding integrity, it has been concluded that commonly used sham rTMS methods appear to adequately conceal treatment allocation, leading to acceptable levels of blinding integrity at study end. Notwithstanding, as we did not formally evaluate blinding integrity in our sample we consider this as a limitation of the study. The fact that in our study all TRD patients were 2-week AD-free before scanning should be considered as a major strength of this study. Finally, in spite that functional connectivity is a unique powerful tool able to increase our knowledge on human brain organization, fcMRI is based on an inherently ambiguous measure reflecting constraints both from static anatomical connectivity and from poorly understood functional coupling changes that are dynamic; physiological data from other sources may be required to confirm and interpret FC findings (Buckner et al., 2013).

In conclusion, our current results confirmed the importance of the sgACC as a key structure implicated in TRD. They agree with our a priori hypotheses that an increased anti-correlation between the sgACC and the (predominantly left) PFC is indicative for a

beneficial clinical outcome of accelerated HF-rTMS. Our findings suggest that the stronger anti-correlation between the sgACC and parts of the left prefrontal cortex (BA 10) could be indicative for beneficial outcome, raising the possibility that baseline rsFC scans examining the FC between these regions in particular could be interesting for predicting clinical effects. When clinically effective, intensified HF-rTMS yields similar effects on FC between the sgACC and parts of the pgPFC cortex as other AD treatment algorithms, such as the more invasive neurostimulation techniques. However, the most fascinating result to report here is that these neurobiological effects in HF-rTMS responders are already present after 4 days of real stimulation**.** Multimodal brain imaging paradigms are needed to substantiate our findings that (accelerated) HF-rTMS treatment designs have the potential to acutely adjust deregulated sgACC neuronal networks in TRD patients.

Acknowledgments

This research was supported by a grant from the Scientific Fund W. Gepts UZBrussel. This work was also supported by the Ghent University Multidisciplinary Research Partnership "The integrative neuroscience of behavioral control". MAV (FWO08/PDO/168) is a postdoctoral fellow of the Research Foundation Flanders (FWO). Preparation of this paper was also supported by Grant BOF10/GOA/014 for a Concerted Research Action of Ghent University (awarded to RDR). No authors have financial disclosures to report.

Conflict of interest statement

None to declare.

References

Amodio DM, Frith CD. 2006. Meeting of minds: the medial frontal cortex and social cognition. Nat Rev Neurosci 7: 268-277.

Amting JM, Greening SG, Mitchell DG. 2010. Multiple mechanisms of consciousness: the neural correlates of emotional awareness. J Neurosci 30: 10039-10047.

Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. 2010. Functional-anatomic fractionation of the brain's default network. Neuron 65: 550-562.

Baeken C, De Raedt R, Van Hove C, Clerinx P, De Mey J, Bossuyt A. 2009. HF-rTMS treatment in medication-resistant melancholic depression: results from 18FDG-PET brain imaging. CNS Spectr 14: 439-448.

Baeken C, Vanderhasselt MA, Remue J, Herremans S, Vanderbruggen N, Zeeuws D, Santermans L, De Raedt R. J Affect Disord. *In press.* Intensive HF-rTMS treatment in refractory medication-resistant unipolar depressed patients. doi: 10.1016/j.jad.2013.07.008.

Berlim MT, Broadbent HJ, Van den Eynde F. 2013. Blinding integrity in randomized sham-controlled trials of repetitive transcranial magnetic stimulation for major depression: a systematic review and meta-analysis. Int. J. Neuropsychopharmacol 16: 1173-1181.

Brett M, Anton J-L, Valabregue R, Poline J-B. 2002. Region of interest analysis using an SPM toolbox [abstract]. Presented at the Eighth International Conference of Functional Mapping of the Human Brain, June 2–6, 2002, Sendai, Japan. Available on CD-ROM in NeuroImage, Vol. 16, No. 2.

Brunoni AR, Valiengo L, Baccaro A, Zanão TA, de Oliveira JF, Goulart A, Boggio PS, Lotufo PA, Benseñor IM, Fregni F. 2013. The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial. JAMA Psychiatry 70: 383-391.

Buckner RL, Krienen FM, Yeo BT. 2013. Opportunities and limitations of intrinsic functional connectivity MRI. Nat Neurosci 16: 832-837.

Burgess PW, Gilbert SJ, Dumontheil I. 2007 a. Function and localization within rostral prefrontal cortex (area 10). Philos. Trans. R. Soc. Lond B Biol Sci 362: 887-899.

Burgess PW, Dumontheil I, Gilbert SJ. 2007 b. The gateway hypothesis of rostral prefrontal cortex (area 10) function. Trends Cogn Sci 11: 290-298.

Carballedo A, Scheuerecker J, Meisenzahl E, Schoepf V, Bokde A, Möller HJ et al. 2011. Functional connectivity of emotional processing in depression. J Affect Disord 134: 272-279.

Cisler JM, James GA, Tripathi S, Mletzko T, Heim C, Hu XP et al. 2013. Differential functional connectivity within an emotion regulation neural network among individuals resilient and susceptible to the depressogenic effects of early life stress. Psychol Med 43: 507-518.

Davey CG, Harrison BJ, Yücel M, Allen NB. 2012 a. Regionally specific alterations in functional connectivity of the anterior cingulate cortex in major depressive disorder. Psychol Med 42: 2071-2081.

Davey CG, Yücel M, Allen NB, Harrison BJ. 2012 b. Task-related deactivation and functional connectivity of the subgenual cingulate cortex in major depressive disorder. Front Psychiatry 3: 14.

Disner SG, Beevers CG, Haigh EA, Beck AT. 2011. Neural mechanisms of the cognitive model of depression. Nat Rev Neurosci 12: 467-477.

Dougherty DD, Weiss AP, Cosgrove GR, Alpert NM, Cassem EH, Nierenberg AA et al. 2003. Cerebral metabolic correlates as potential predictors of response to anterior cingulotomy for treatment of major depression. J Neurosurg 99: 1010-1017.

Drevets WC, Price JL, Simpson JR Jr, Todd RD, Reich T, Vannier M et al. 1997. Subgenual prefrontal cortex abnormalities in mood disorders. Nature 386: 824-827.

Drevets WC, Price JL, Furey ML. 2008 a. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Structure and Function 213: 93-118.

Drevets WC, Savitz J, Trimble M. 2008 b. The subgenual anterior cingulate cortex in mood disorders. CNS Spect 13: 663-681.

Etkin A, Egner T, Kalisch R. 2011. Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn Sci 15: 85-93.

Fava, M. 2003. The role of the serotonergic and noradrenergic neurotransmitter systems in the treatment of psychological and physical symptoms of depression. J Clin Psychiatry 64: 26-29.

Fitzgerald PB, Daskalakis ZJ. 2012. A practical guide to the use of repetitive transcranial magnetic stimulation in the treatment of depression. Brain Stimul 5: 287-296.

Fox MD, Greicius M. 2010. Clinical applications of resting state functional connectivity. Front Syst Neurosci 4: 19.

Fox MD, Buckner RL, White MP, Greicius MD, Pascual-Leone A. 2012 a. Efficacy of Transcranial Magnetic Stimulation Targets for Depression Is Related to Intrinsic Functional Connectivity with the Subgenual Cingulate. Biol Psychiatry 72: 595-603.

Fox MD, Halko MA, Eldaief MC, Pascual-Leone A. 2012 b. Measuring and manipulating brain connectivity with resting state functional connectivity magnetic resonance imaging (fcMRI) and transcranial magnetic stimulation (TMS). Neuroimage 62: 2232-2243.

George MS, Nahas Z, Kozol FA, Li X, Yamanaka K, Mishory A et al. 2003. Mechanisms and the current state of transcranial magnetic stimulation. CNS Spectr 8, 496-514.

George MS, Post RM. 2011. Daily left prefrontal repetitive transcranial magnetic stimulation for acute treatment of medication-resistant depression. Am J Psychiatry 168: 356-364.

Gershon AA, Dannon PN, Grunhaus L. 2003. Transcranial magnetic stimulation in the treatment of depression. Am J Psychiatry 160: 835-845.

Greicius MD, Flores BH, Menon V, Glover GH, Solvason. 2007. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biol Psychiatry 62: 429-437.

Guinjoan SM, Mayberg HS, Costanzo EY, Fahrer RD, Tenca E, Antico J et al. 2010. Asymmetrical contribution of brain structures to treatment-resistant depression as illustrated by effects of right subgenual cingulum stimulation. J Neuropsychiatry Clin Neurosci 22: 265-277.

Hadley D, Anderson BS, Borckardt JJ, Arana A, Li X, Nahas Z, George MS. 2011. Safety, tolerability, and effectiveness of high doses of adjunctive daily left prefrontal repetitive transcranial magnetic stimulation for treatment-resistant depression in a clinical setting. J ECT 27: 18-25.

Hamani C, Mayberg H, Stone S, Laxton A, Haber S, Lozano AM. 2011. The subcallosal cingulate gyrus in the context of major depression. Biol Psychiatry 69: 301-308.

Hamilton M. 1967. Development of a rating scale for primary depressive illness. Br J Soc & Clin Psychology 6: 278-296.

Hamilton JP, Chen G, Thomason ME, Schwartz ME, Gotlib IH. 2011. Investigating neural primacy in Major Depressive Disorder: multivariate Granger causality analysis of resting-state fMRI time-series data. Mol Psychiatry 16: 763-772.

Holtzheimer PE 3rd, Russo J, Claypoole KH, Roy-Byrne P, Avery DH. 2004. Shorter duration of depressive episode may predict response to repetitive transcranial magnetic stimulation. Depress Anxiety 19: 24-30.

Holtzheimer PE 3rd, McDonald WM, Mufti M, Kelley ME, Quinn S, Corso G et al. 2010. Accelerated repetitive transcranial magnetic stimulation for treatment-resistant depression. Depress Anxiety 27: 960-963.

Holtzheimer PE, Mayberg HS. 2011. Stuck in a rut: rethinking depression and its treatment. Trends Neurosci 34: 1-9.

Johansen-Berg H, Gutman DA, Behrens TE, Matthews PM, Rushworth MF, Katz E et al. 2008. Anatomical connectivity of the subgenual cingulate region targeted with deep brain stimulation for treatment-resistant depression. Cereb Cortex 18: 1374-1383.

Kito S, Fujita K, Koga Y. 2008. Changes in regional cerebral blood flow after repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex in treatment-resistant depression. J Neuropsychiatry Clin Neurosci 20: 74-80.

Kito S, Hasegawa T, Koga Y. 2012. Cerebral blood flow ratio of the dorsolateral prefrontal cortex to the ventromedial prefrontal cortex as a potential predictor of treatment response to transcranial magnetic stimulation in depression. Brain Stimul 5: 547-553.

Knoch D, Treyer V, Regard M, Muri RM, Buck A, Weber B. 2006. Lateralized and frequency-dependent effects of prefrontal rTMS on regional cerebral blood flow. Neuroimage 3: 641-648.

Koch W, Teipel S, Mueller S, Buerger K, Bokde AL, Hampel H et al. 2010. Effects of aging on default mode network activity in resting state fMRI: does the method of analysis matter? Neuroimage 51: 280-287.

Marchetti I, Koster EH, Sonuga-Barke EJ, De Raedt R. 2012. The Default Mode Network and Recurrent Depression: A Neurobiological Model of Cognitive Risk Factors. Neuropsychol Rev 22: 229-251.

Mayberg HS, Brannan SK, Mahurin RK, Jerabek PA, Brickman JS, Tekell JL et al. 1997. Cingulate function in depression: a potential predictor of treatment response. Neuroreport 8: 1057-1061.

Mayberg HS. 2003. Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. Br Med Bull 65:193-207.

Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C et al. 2005. Deep brain stimulation for treatment-resistant depression. Neuron 45: 651-660.

Mottaghy FM, Keller CE, Gangitano M, Ly J, Thall M, Parker JA et al. 2002. Correlation of cerebral blood flow and treatment effects of repetitive transcranial magnetic stimulation in depressed patients. Psychiatry Res 115: 1-14.

Nemeroff, CB. 2007a. Prevalence and management of treatment-resistant depression. J Clin Psychiatry 68: 17-25.

Nemeroff, CB. 2007b. The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J Psychiatr Res 41: 189-206.

Nobler MS, Oquendo MA, Kegeles LS, Malone KM, Campbell CC, Sackeim HA et al. 2001. Decreased regional brain metabolism after ect. Am J Psychiatry 158: 305-308.

Ochsner KN, Gross JJ. 2005. The cognitive control of emotion. Trends Cogn Sci 9: 242-249.

Padberg F, George MS. 2009. Repetitive transcranial magnetic stimulation of the prefrontal cortex in depression. Exp Neurol 219: 2-13.

Paus T, Castro-Alamancos MA, Petrides M. 2001. Cortico-cortical connectivity of the human mid-dorsolateral frontal cortex and its modulation by repetitive transcranial magnetic stimulation. Eur J Neurosci 14: 1405-1411.

Paus T, Barrett J. 2004. Transcranial magnetic stimulation (TMS) of the human frontal cortex: implications for repetitive TMS treatment of depression. J Psychiatry Neurosci 29: 268-279.

Peleman K, Van Schuerbeek P, Luypaert R, Stadnik T, De Raedt R, De Mey J et al. 2010. Using 3D-MRI to localize the dorsolateral prefrontal cortex in TMS research. World J Biol Psychiatry 11: 425-430.

Pizzagalli D, Pascual-Marqui RD, Nitschke JB, Oakes TR, Larson CL, Abercrombie HC et al. 2001. Anterior cingulate activity as a predictor of degree of treatment response in major depression: evidence from brain electrical tomography analysis. Am J Psychiatry 158: 405-415.

Pizzagalli DA. 2011. Frontocingulate dysfunction in depression: toward biomarkers of treatment response. Neuropsychopharmacology 36: 183-206.

Pollmann S, Manginelli AA. 2009. Anterior prefrontal involvement in implicit contextual change detection. Front Hum Neurosci 3: 28.

Price JL, Drevets WC. 2010. Neurocircuitry of mood disorders. Neuropsychopharmacology 35: 192-216.

Price JL, Drevets WC. 2012. Neural circuits underlying the pathophysiology of mood disorders. Trends Cogn Sci 16: 61-71.

Ramnani N, Owen AM. 2004. Anterior prefrontal cortex: insights into function from anatomy and neuroimaging. Nat Rev Neurosci 5: 184-194.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A; Safety of TMS Consensus Group. 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 120: 2008-2039.

Rush AJ, Thase ME, Dubé S. 2003. Research issues in the study of difficult-to-treat depression. Biol Psychiatry 53: 743-753.

Schutter DJ. 2009. Antidepressant efficacy of high-frequency transcranial magnetic stimulation over the left dorsolateral prefrontal cortex in double-blind sham-controlled designs: a meta-analysis. Psychol Med 39, 65-75.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation

of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 20: 22-57.

Sheline YI, Price JL, Yan Z, Mintun MA. 2010. Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. Proc Natl Acad Sci U S A 107: 11020-11025.

Shelton RC, Osuntokun O, Heinloth AN, Corya SA. 2010. Therapeutic options for treatment-resistant depression. CNS Drugs 24: 131-161.

Smith R, Fadok RA, Purcell M, Liu S, Stonnington C, Spetzler RF et al. 2011. Localizing sadness activation within the subgenual cingulate in individuals: a novel functional MRI paradigm for detecting individual differences in the neural circuitry underlying depression. Brain Imaging Behav 5: 229-239.

Steele JD, Christmas D, Eljamel MS, Matthews K. 2008. Anterior cingulotomy for major depression: clinical outcome and relationship to lesion characteristics. Biol Psychiatry 63: 670-677.

Van Strien JW & Van Beek S. 2000. Ratings of emotion in laterally presented faces: sex and handedness effects. Brain Cogn 44: 645-652.

Veer IM, Beckmann CF, van Tol MJ, Ferrarini L, Milles J, Veltman DJ et al. 2010. Whole brain resting-state analysis reveals decreased functional connectivity in major depression. Front Syst Neurosci 4: 41.

Wang L, Hermans D F, Hickie I B, Lagopoulos J. 2012. A systematic review of resting-state functional-MRI studies in major depression. J Affect Disord 142: 6-12.

Ward MP, Irazoqui PP. 2010. Evolving refractory major depressive disorder diagnostic and treatment paradigms: toward closed-loop therapeutics. Front Neuroengineering 31: 3-7.

Wassermann EM. 1998. Risk and Safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of repetitive transcranial Magnetic Stimulation. Electroencephalogr Clin Neurophysiol 108: 1-16.

Wendelken C, Nakhabenko D, Donohu, SE, Carter CS, Bunge SA. 2008. "Brain is to thought as stomach is to ??": investigating the role of rostrolateral prefrontal cortex in relational reasoning. J Cogn Neurosci 20: 682-693.

Wu JT, Wu HZ, Yan CG, Chen WX, Zhang HY, He Y et al. 2011. Aging-related changes in the default mode network and its anti-correlated networks: a resting-state fMRI study. Neurosci Lett 504: 62-67.

Zeeuws D, De Rycker K, De Raedt R, De Beyne M, Baeken C, Vanderbruggen N. 2011. Intensive high-frequency repetitive transcranial magnetic stimulation treatment in an electroconvulsive shock therapy-resistant bipolar I patient with mixed episode. Brain Stimul 4: 46-49.

Zobel A, Joe A, Freymann N, Clusmann H, Schramm J, Reinhardt M, Biersack HJ, Maier W, Broich K. 2005. Changes in regional cerebral blood flow by therapeutic vagus nerve stimulation in depression: an exploratory approach. Psychiatry Res. 139:165-179.

Demographic data and behavioral results.						
	All patients	Responders After week one (n=2)	Responders After week two (n=5)	Total Responders (n=7)	Total Non-responders (n=13)	*p*-values *
Age (years)	49 (13)	42 (13)	41 (16)	42 (14)	53 (11)	0.06
Gender (male:female)	7:20	0: 2	2: 3	2:5	5:13	0.66
HDRS T_0	26 (6)	24 (7)	26 (5)	25 (5)	26 (7)	0.78
HDRS T_1	20 (7)	11 (2)	18 (7)	16 (3)	23 (7)	0.06
HDRS T_2	18 (9)	7 (1)	8 (2)	7 (2)	23 (5)	<0.01

Table 1. Mean and SD for the basic demographic quantities Age and Gender and the results for the HDRS scores (17- item Hamilton depression rating scale) of the subject categories. The *p* - values report the significance levels reached for the T or X^2 tests (as applicable) comparing responders and non-responders. The

significance threshold was set at $p < 0.05$ for all analyses. * Represents p-values only represent differences between the group of responders and non-responders.

(a) Two-way ANOVA						
	Cluster size	**Anatomical region**	**Hemisphere**	**BA**	**F-value**	**Peak coordinates (x,y,z) (mm)**
Interaction effects						
Cluster	435	Superior Medial Frontal Gyrus	Left / Right	-	3.62	(0, 60, 21)

(a) Post-hoc tests comparing responders vs. non-responders						
	Cluster size	**Anatomical region**	**Hemisphere**	**BA**	**T-value**	**Peak coordinates (x,y,z) (mm)**
Baseline						
Cluster 1	108	Superior Medial Frontal Gyrus	Left / Right	10	-3.96	(0, 4,8 30)
Cluster 2	47	Superior Frontal Gyrus	Left	10	-3.89	(-24, 51, 27)
After treatment						
Cluster	298	Anterior Cingulate gyrus / Superior Medial Frontal Gyrus	Right	32 / 10	3.98	(6, 45, 9)

Table 2. (a) Results of the two-way ANOVA whole brain analysis of the sgACC rsFC, showing the areas with significant Response x Treatment interaction. (b) Post-hoc comparison of responders vs. non-responders, showing significant T-test clusters at baseline and after HF-rTMS treatment. The significance threshold was set at $p < 0.05$ for all analyses.

Figure legends

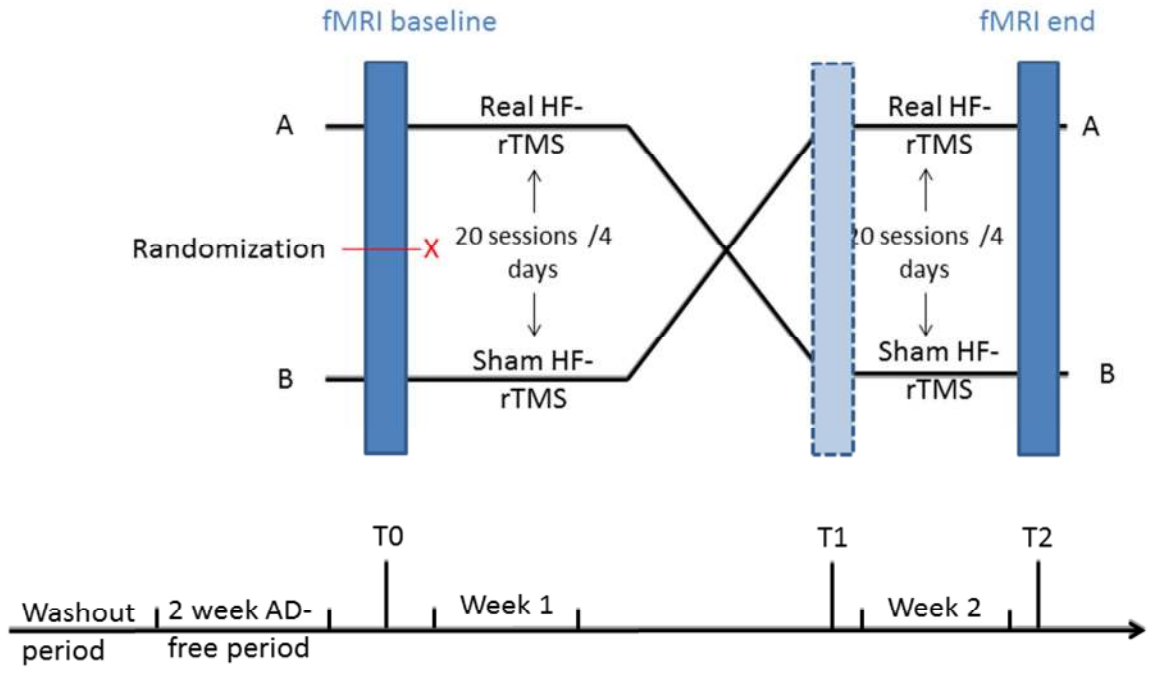

Figure 1. Flow chart of the experimental HF-rTMS treatment procedure.

After a washout period, all TRD (treatment resistant depression) patients are at least two weeks antidepressant (AD) free before they undergo the first (baseline) functional connectivity MRI (fcMRI) scan at time T_0 (on a Monday afternoon). Hereafter patients are randomly divided into two groups of 10 to receive 20 sessions of real or sham HF-rTMS treatment respectively. This treatment is spread over the four succeeding afternoons (5 daily sessions on Tuesday, Wednesday, Thursday and Friday). In the second week, strictly the same treatment schedule is followed but with a change of stimulation: line AB= a TRD patient who first received real HF-rTMS now receives sham; line BA= a patient who first received sham treatment now receives real HF-rTMS. A second fcMRI scan (fcMRI end) is performed exactly 1 week after the first week (time T_1) and a third scan exactly after 2 weeks (time T_2), always on a Monday afternoon. At T_0, T_1 and T_2 all patients are assessed using the Hamilton Depression Rating Scale (HDRS).

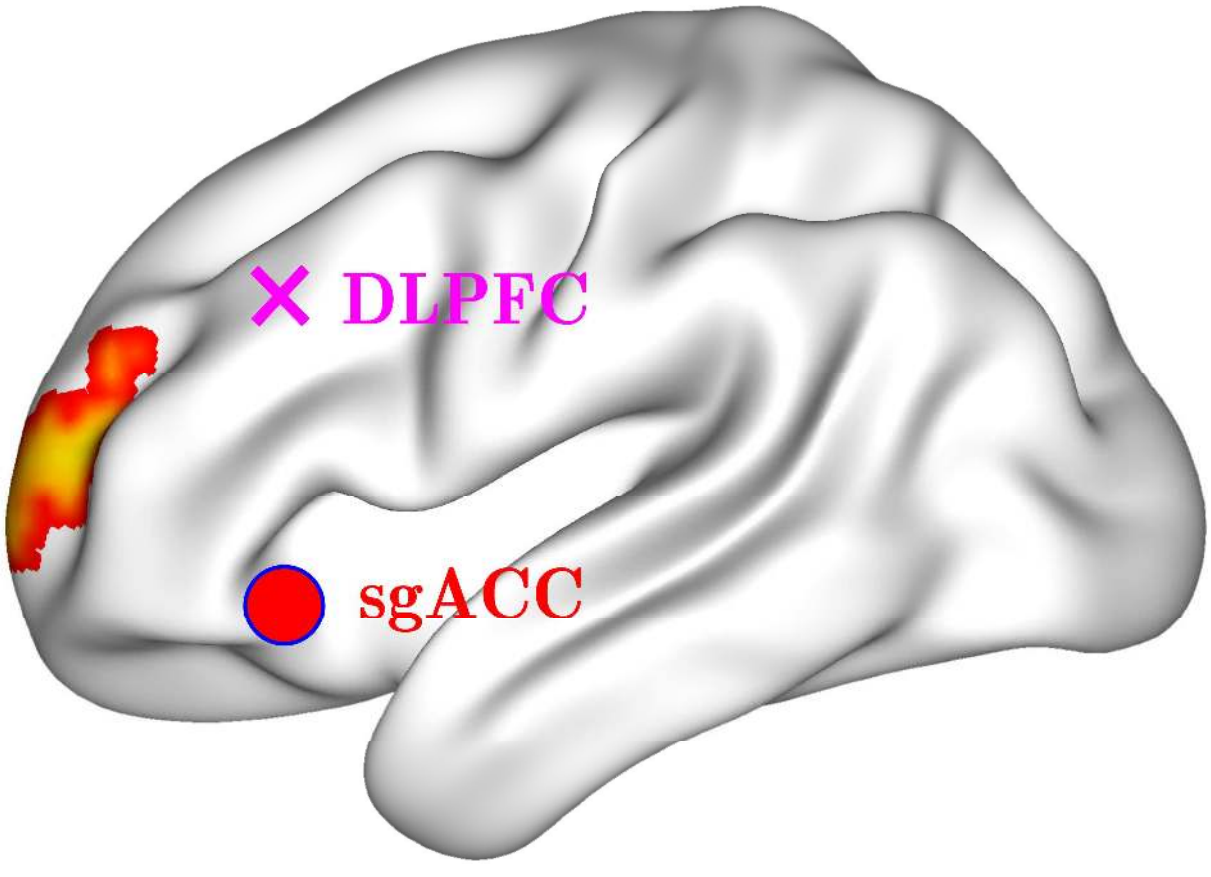

Figure 2. Sagittal glass brain image depicting the significant Response x Treatment interaction cluster result.

The red-shaded areas resulted from a two-way ANOVA whole brain analysis of the sgACC rsFC, showing a significant Response x Treatment-interaction region located in the superior medial frontal gyrus.

The red sphere represents the seed region, a 6-mm-diameter sphere centered around MNI coordinates: x= 1, y= 25, z= -11, subgenual anterior cingulate cortex (sgACC). The purple sphere is centered on the target area in the dorsolateral prefrontal cortex (DLPFC, BA 46/9, MNI coordinates: x= −45, y= 30, z= 31).

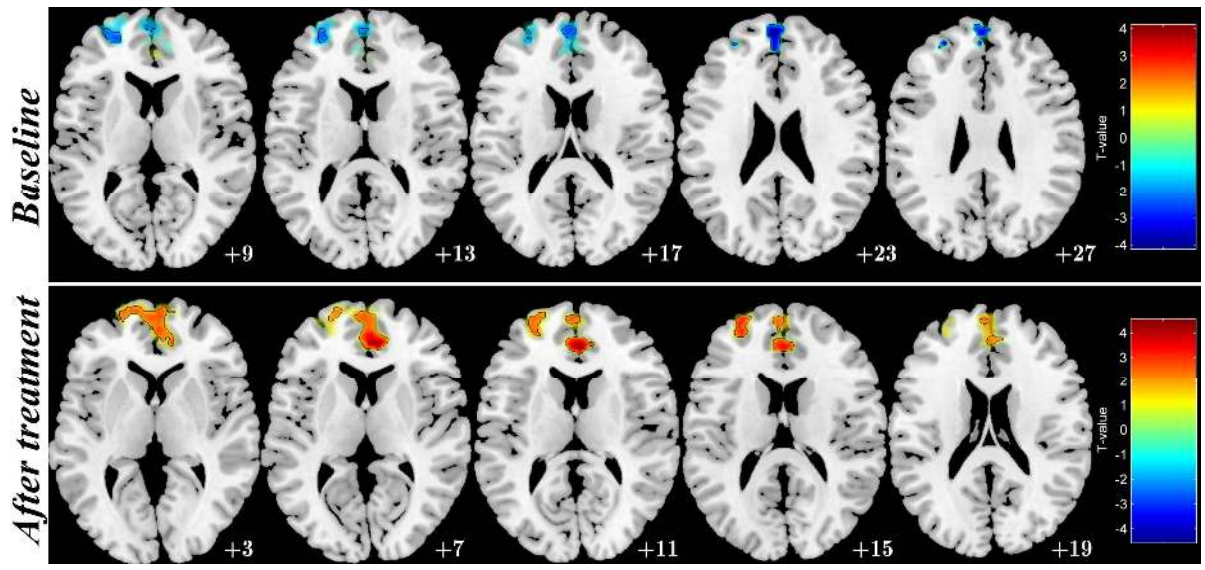

Figure 3. Transversal slices depicting the Response x Treatment interaction cluster result

Results of the post hoc T-tests comparing the sgACC rsFC of responders and non-responders at baseline (top row) and after treatment (bottom row). The significant Response x Treatment interaction cluster was used as mask. Blue-shaded regions (predominantly left prefrontal cortex (BA 10)) exhibit significantly stronger sgACC rsFC anti-correlation in responders compared to non-responders. Red-shaded regions (parts of the perigenual prefrontal cortex (BA 32/10)) exhibit significantly weaker sgACC rsFC anti-correlation in responders compared to non-responders.

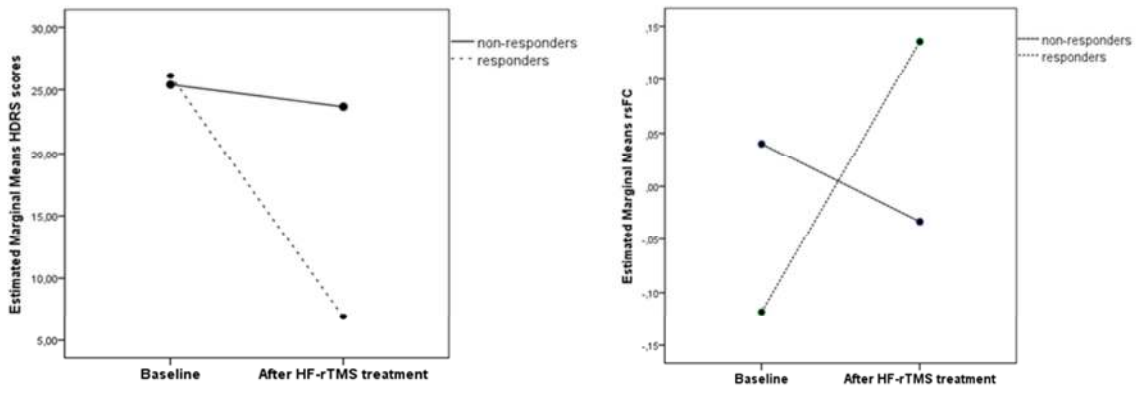

Figure 4. Graphical representation of the interaction between the two factors Response (positive, negative) and Treatment (baseline, post-treatment) of the two-way ANOVA containing age as covariate, for the HDRS (Hamilton Depression Rating Scale; on the left) scores and the rsFC (resting state functional connectivity; on the right).