Neurobiological and clinical effects of fNIRS-controlled rTMS in patients with panic disorder/agoraphobia during cognitive-behavioural therapy

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ABSTRACT

Background: A relevant proportion of patients with panic disorder (PD) does not improve even though they receive state of the art treatment for anxiety disorders such as cognitive-behavioural therapy (CBT). At the same time, it is known, that from a neurobiological point of view, PD patients are often characterised by prefrontal hypoactivation. Intermittent Theta Burst Stimulation (iTBS) is a non-invasive type of neurostimulation which can modulate cortical activity and thus has the potential to normalise prefrontal hypoactivity found in PD. We therefore aimed at investigating the effects of iTBS as an innovative add-on to CBT in the treatment for PD.

Methods: In this double-blind, bicentric study, 44 PD patients, randomised to sham or verum stimulation, received 15 sessions of iTBS over the left prefrontal cortex (PFC) in addition to 9 weeks of group CBT. Cortical activity during a cognitive as well as an emotional (Emotional Stroop) paradigm was assessed both at baseline and post-iTBS treatment using functional near-infrared spectroscopy (fNIRS) and compared to healthy controls.

Results: In this manuscript we only report the results of the emotional paradigm; for the results of the cognitive paradigm please refer to Deppermann et al. (2014).

During the Emotional Stroop test, PD patients showed significantly reduced activation to panic-related compared to neutral stimuli for the left PFC at baseline. Bilateral prefrontal activation for panic-related stimuli significantly increased after verum iTBS only. Clinical ratings significantly improved during CBT and remained stable at follow-up. However, no clinical differences between the verum- and sham-stimulated group were identified, except for a more stable reduction of agoraphobic avoidance during follow-up in the verum iTBS group.

Limitations: Limitations include insufficient blinding, the missing control for possible state-dependent iTBS effects, and the timing of iTBS application during CBT.

Conclusion: Prefrontal hypoactivity in PD patients was normalised by add-on iTBS. Clinical improvement of anxiety symptoms was not affected by iTBS.

1. Introduction

With a 12-month prevalence of 2–3% (Kessler et al., 2006; Wittchen et al., 2011), panic disorder (PD) and comorbid agoraphobia represent a massively impairing anxiety disorder (Barlow, 2002) posing a substantial economic burden (Zaubler and Katon, 1998), and high

Abbreviations: ANOVA, analysis of variance; CAQ, Cardiac Anxiety Questionnaire; CBSI, correlation-based signal improvement; CBT, cognitive-behavioural therapy; ER, error rate; fNIRS, functional near-infrared spectroscopy; HAM-A, Hamilton Anxiety Rating Scale; HHb, deoxyhemoglobin; iTBS, intermittent Theta Burst Stimulation; LOCF, last observation carried forward; O2Hb, oxyhemoglobin; PD, panic disorder; PAS, Panic and Agoraphobia Scale; PFC, prefrontal cortex; RM-ANOVA, repeated-measures analysis of variance; ROI, region of interest; RT, reaction time; rTMS, repetitive Transcranial Magnetic Stimulation

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comorbidity and/or chronicity are frequently observed in this group of patients (Roy-Byrne et al., 2006). Fortunately, effective treatment options exist, as cognitive-behavioural therapy (CBT) has been proven effective in numerous randomised controlled studies (Bandelow et al., 2007; Hofmann and Smits, 2008; Schmidt and Keough, 2010). Moreover, pharmacotherapy has been confirmed to be beneficial in the treatment of PD with/without agoraphobia (Bandelow et al., 2008). However, up to one third of patients do not respond sufficiently to either approach (Diemer et al., 2010; Taylor et al., 2012). Several factors contributing to this phenomenon have been observed, e.g. disorder duration (Scheibe and Albus, 1996; Slaap and den Boer, 2001). Thus, despite a wide range of treatments available, improved therapeutic strategies for PD and agoraphobia are still needed.

From a neurobiological point of view of PD, alterations of the “fear network” in terms of hyperactivity of subcortical structures such as the amygdala have been consistently observed (cf. de Carvalho et al., 2010). Concurrently, a number of imaging studies have shown hypo-activation of the lateral prefrontal cortex, which is indirectly linked to the amygdala and is known to be critically involved in voluntary emotion regulation and cognitive control (Urry et al., 2006; Kent and Rauch, 2003; but see Dresler et al., 2013 for a comprehensive review). Since CBT works by changing problematic cognitions and prompting inhibitory learning (Craske et al., 2014), hypothetically, on a neurobiological basis, these effects of CBT should be associated with increased prefrontal activation which has in fact been shown in a number of studies (for a review see Clark and Beck, 2010). By implication, one could further conclude that directly enhancing prefrontal activation patterns in addition to CBT might enhance CBT outcome.

Based on the principle of electro-magnetic induction, repetitive Transcranial Magnetic Stimulation (rTMS) is capable of modulating cortical activity locally and non-invasively (Wassermann and Zimmermann, 2012). RTMS applied to the prefrontal cortex has been shown to exert antidepressant effects in several sham-controlled trials (Schutter, 2009; Berlim et al., 2013), however, inconsistent findings exist (Herwig et al., 2007). As a potential treatment option for anxiety disorders, the technique has so far been less investigated (Paes et al., 2011; Zwanzger et al., 2009). Although promising results have been demonstrated in small controlled trials, open studies and case reports (Mantovani et al., 2007; Paes et al., 2011; Zwanzger et al., 2009; Zwanzger et al., 2002; Dresler et al., 2009), again so far the findings are not conclusive and further controlled studies are needed to determine the optimal stimulation characteristics (Prasko et al., 2007) To increase cortical activity, the rTMS protocol intermittent Theta Burst Stimulation (iTBS) is recommended (Huang et al., 2005).

To evaluate cortical effects of neurobiological interventions, functional near-infrared spectroscopy (fNIRS) provides a non-invasive optical imaging technique that applies near-infrared light to measure task-related alterations of oxygenated and deoxygenated haemoglobin concentrations (Ferrari and Quaresima, 2012; Ehlis et al., 2014). Advantages compared to fMRI-investigations are considerable: fNIRS devices are mobile and allow for a more comfortable investigation without a potentially anxiety-inducing scanner environment, which might be particularly favourable for patients with claustrophobic difficulties (cf. Ohta et al., 2008).

In the present pilot study, we aimed at investigating, whether iTBS, applied concurrently to group CBT for PD, normalises prefrontal hypoactivity in terms of a “trans-situat characteristic” in this group of patients but also during specific fear-relevant situations. Do to so, we applied a cognitive task as well as an emotional task. Whereas the results of the cognitive task and the corresponding clinical data collected during the first three weeks of iTBS treatment have been published in Deppermann et al. (2014), this manuscript focuses on the results of the emotional paradigm (Emotional Stroop task) and the clinical data which was collected over the whole time course of CBT. More specifically, the following hypotheses were tested: (1) PD/agoraphobia patients are characterised by prefrontal hypoactivation, as assessed by fNIRS, during a task that requires emotion regulation and cognitive control (Emotional Stroop task) compared to controls. (2) CBT and add-on iTBS normalises these activation patterns and (3) improves clinical symptoms. (4) Changes in fNIRS patterns are correlated with treatment efficacy.

2. Materials and methods

Inclusion criteria, implementation of fNIRS and iTBS application were identical to the procedures described in Deppermann et al. (2014) but, for more clarity, will be delineated again in the following sections.

2.1. Participants

The study included 44 patients, aged 18–65 years and diagnosed with PD with/without agoraphobia according to the DSM-IV-TR (American Psychiatric Association, 2000). PD with/without agoraphobia was diagnosed by experienced clinical psychologists with the Structured Clinical Interview for DSM-IV, Axis I Disorders (SCID-I; First et al., 1996; Wittchen et al., 1997). In the PD group, comorbid psychiatric disorders (except for bipolar or psychotic disorder, borderline personality disorder, acute substance abuse disorders and acute suicidality) were no exclusion criteria and the intake of psychopharmacological medication like selective serotonin (noradrenaline) reuptake inhibitors was permitted if the dosage had been kept stable for at least three weeks prior to baseline assessment.

23 healthy controls with no family history of mental disorders and no current or past mental, somatic or organic brain disorder were included. Groups did not differ with respect to gender, age, years of education, handedness, comorbid depression or duration of illness (Table 1). After a comprehensive study description, written informed consent was obtained. A clinical trial registration did not take place but the study was approved by the Ethics Committees of the Universities of Muenster and Tuebingen. All procedures were in accordance with the Declaration of Helsinki in its latest version.

2.2. Design

This multicentre study combined a 9-week CBT group intervention with a sham-controlled iTBS augmentation within the first 3 weeks of CBT. Patients diagnosed with PD with/without agoraphobia were randomised to either sham or verum iTBS. Enrolment took place between 01/2011 and 07/2013. Patients and therapists were blinded to iTBS group assignment (Fig. 1).

2.3. CBT

CBT (based on Margraf and Schneider (1990) and Schneider and Margraf (1998)) was conducted as a standardised treatment by trained clinical psychologists, who were continually supervised by experienced clinical psychotherapists. It was administered in a 9-week group setting (except for session 6) with a maximum of 6 patients/group. Two booster sessions took place after 3 and 6 months, respectively. Sessions lasted 1 ½ hours each, respectively (Fig. 1).

2.4. iTBS

After randomisation, a (sham) iTBS protocol (Huang et al., 2005) was applied over the left PFC in 15 daily sessions which always took place at the same time during the day for each individual patient but could vary between patients depending on their available free time during the first three weeks of CBT. We used a figure-of-eight coil (MCF- 656, 2 × 75 mm diameter, n = 34, MAGSTIM 9925-00, 2 × 70 mm, n = 9) using a MagOption/MagPro × 100 stimulator (MagVenture, Denmark, n = 35), and a MAGSTIM RAPID2 T/N 3567-23-02 stimulator (n = 9), respectively. The rTMS coil was placed over electrode
Table 1
Baseline sample characteristics.

|                  | Verum (14) | Sham (12) | Controls (19) | Statistics | Post-hoc |
|------------------|------------|-----------|---------------|------------|----------|
| Number in sample | 22         | 22        | 23            |            |          |
| Mean age in years (range) | 37.6 (19–63) | 36.3 (22–56) | 33.4 (19–64) | F_{2,66} = 0.807, p = 0.45 |
| % women          | 59 (50)    | 64 (75)   | 61 (63)       | X^2 = 0.097, p = 0.95 |
| Handedness (number of right-handed subjects) | 20 (13)    | 21 (12)   | 20 (16)       | x = 1.037, p = 0.87 |
| First Language   | 19 (13)    | 19 (11)   | 22 (18)       | x = 2.74, p = 0.64 |
| Mean years of education (SD) | 12.1 (1.7) | 12.4 (2.0) | 12.5 (1.1)    | F_{2,66} = 0.33, p = 0.72 |
| Mean duration of illness in months (range) | 92 (1–372) | 84 (1–336) | (109.8 (18.372) | F_{1,41} = 0.084, p = 0.77 |
| Comorbid depression | 8 (4) | 6 (2) | – | x = 0.56, p = 0.92 |
| HAM-A – total (SD) | 22.41 (8.97) | 20.3 (7.1) | 3.90 (3.35) | F_{2,66} = 50.49, p < 0.001 |
| Self-rated PAS total (SD) | 20.76 (7.76) | 20.52 (8.10) | 0.22 (1.04) | F_{2,66} = 75.64, p < 0.001 |
| CAQ – total (SD) | 1.63 (0.71) | 1.36 (0.51) | 0.33 (0.20) | F_{2,66} = 39.95, p < 0.001 |

CAQ: Cardiac Anxiety Questionnaire; HAM-A: Hamilton Anxiety Rating Scale; HC: healthy controls; PAS: Panic and Agoraphobia Scale; S: sham group; SD: standard deviation; V: verum group; values in parentheses indicate results for the subgroup used for analyses of the behavioural data during the Emotional Stroop task. For all questionnaires, higher scores indicate higher severity of symptoms. For PAS, the median for PD-patients is reported to be 23 [Bandelow, 1997].

position F3 (left dorsolateral PFC) of the international 10–20 EEG system (Herwig et al., 2003). In order to adjust the stimulation intensity to the individual cortical excitability, the participants’ resting motor threshold was defined prior to each iTBS application and stimulation intensity was set to 80% of it.

As a manipulation check, after all 15 iTBS sessions were completed, the participants were asked which stimulation (verum or sham) they believed they had received.

2.5. Outcome measures

2.5.1. Emotional Stroop task

The Emotional Stroop task consisted of 15 panic-related and 15 neutral words presented in red, green, yellow and blue. The words belonging to the two conditions did not significantly differ with regard to the number of letters, syllables and frequency in spoken/written language. Furthermore, they had already been used in prior studies (e.g., Dresler et al., 2012). Participants had to indicate the word colour independent of its meaning via button press. It is assumed that emotional, in contrast to neutral, words bind more attention due to
emotional interference, thereby increasing reaction times (RTs) and error rates (ERs) for emotional words. For panic-related words, this effect should be more pronounced in PD patients (Dresler et al., 2012).

All 120 trials were presented in randomised order on a black LCD screen. A fixation cross (500 ms) preceded each stimulus (1500 ms), while the inter-trial intervals (4000–8000 ms) were randomly jittered. We assessed RTs and ERs as indices of emotional interference.

2.5.2. fNIRS measures
fNIRS measurements were conducted using the ETG-4000 Optical Topography System (Hitachi Medical Co., Japan). The probe set consisted of 52 channels arranged in a 3 × 11 optode array (16 photodetectors and 17 light emitters). It was placed with its central optode of the lowest row on FPz stretching out towards T3 and T4, respectively, according to the 10–20 international EEG system (Jasper, 1958).

We recorded changes of the concentration of O_{2}Hb and HHb relative to the individual resting baseline during the Emotional Stroop task for the two conditions neutral words and panic-related words, respectively. The sampling frequency was set to 10 Hz. Measurements took place at baseline just before the beginning of the treatment period (within a range of 48 h before the first iTBS session) as well as after the completion of all 15 iTBS sessions. In order to avoid the measurement of acute iTBS effects, the post measurement was set to be performed after at least 12 h past the last iTBS session (please also refer to Fig. 1).

2.5.3. Clinical outcome measures
Quantitative psychometric assessment was administered at baseline, day 7 (iTBS-7), day 14 (iTBS-14), day 21 (post-iTBS), the end of CBT (post-CBT, week 9), and at 3-month and 6-month follow-up after CBT (Fig. 1). The following questionnaires were used:

- The Panic and Agoraphobia Scale (PAS; Bandelow, 1997) consists of an observer-rated and a self-rated questionnaire assessing symptoms of PD with or without agoraphobia with reasonable reliability and validity (Bandelow, 1997). Each item scores from 0 to 4, with higher scores indicating higher symptom severity. We assessed the total score indicating global severity on both the observer-rated and the self-rated questionnaires, as well as 5 subscores per questionnaire: a) panic attacks, b) agoraphobic avoidance, c) anticipatory anxiety, d) disability and e) worries about health (Bandelow, 1997).

- The Hamilton Anxiety Rating Scale (HAM-A; Hamilton, 1996) is an observer-based, clinical interview assessing a comprehensive range of anxiety symptoms. Beside a total score, the subscales “somatic anxiety” and “psychic anxiety” can be calculated. Higher scores indicate a stronger severity.

- The Cardiac Anxiety Questionnaire (CAQ; Eifert et al., 2000; Hoyer et al., 2005) is a self-report questionnaire with good reliability and validity, designed to assess heart focused anxiety (Eifert et al., 2000; Hoyer et al., 2005). Each item scores from 0 to 4 with higher scores indicating stronger symptoms. Beside a total score, 3 subscales (fear, avoidance, attention) can be calculated.

2.6. Data preparation
Matlab was used to correct for fNIRS signal changes that were not directly due to functional changes in haemoglobin concentration related to the attended tasks and included the following steps: the data was filtered with a high pass of 0.03 and a low pass of 0.5 Hz, manual interpolation of channels which clearly displayed technical artefacts according to a Gaussian distribution (circumjacent channels were taken more into account), a correlation-based signal improvement (CBSI) procedure according to Cui et al. (2010), automatic Gaussian interpolation for channels where the within-subject variance exceeded four. Due to technical problems, complete data sets were only available from n = 20 verum-stimulated patients, n = 21 sham-stimulated patients, and n = 21 healthy controls. The data of the remaining participants were segmented channel-wise in an event-related manner. A time frame of 0–16 s after stimulus onset was extracted and adjusted for linear drifts and baseline. The resulting averaged amplitude integrals (4–10 s after stimulus onset) were taken as the basis for statistical analyses.

For the data of the clinical assessment (HAM-A, PAS, CAQ), a last observation carried forward analysis (LOCF) was applied, if drop-outs or complete omissions of questionnaires between any times of measurement occurred. If there were questionnaire items missing, missing values (if < 10%) were substituted by the mean value of the subject on the relevant scale.

2.7. Regions of interest (ROI)
To assess the effects of the stimulus-related oxygenation changes as well as iTBS treatment, regions of interest (ROIs) were defined a priori. This was done in agreement with current findings on Emotional Stroop paradigms which are known to activate prefrontal areas (such as our site of iTBS application) as the major neural correlate of cognitive control (Tupak et al., 2013; Zhang et al., 2011; Dresler et al., 2012). The channels, including the left and right PFC ROIs, were chosen with respect to a virtual registration procedure described by Tsuzuki et al. (2007), Singh et al. (2005), Rorden and Brett (2000) and Lancaster et al. (2000) (Fig. 2). In order to additionally verify that the expected activation changes were unique to the predefined ROIs, a control “non-ROI” comprising all temporal channels was defined.

2.8. Statistical analyses
Baseline sample characteristics were tested with one-way ANOVAs,
3.1. Sample characteristics

|                  | Sham       | Verum      | Controls |
|------------------|------------|------------|----------|
| RTs (ms)         |            |            |          |
| ERs              |            |            |          |
| Panic-related    | Baseline   | 772 (122)  | 800 (80) |
|                  | 3.8 (0.8)  | 4.0 (1.0)  | 4.2 (1.5) |
| Post-iTBS        | 808 (110)  | 812 (90)   | 800 (102) |
|                  | 4.1 (1.4)  | 4.6 (1.7)  | 5.4 (1.6) |
| Neutral          | Baseline   | 771 (111)  | 799 (80) |
|                  | 0.5 (0.8)  | 2.0 (1.6)  | 1.8 (1.4) |
| Post-iTBS        | 802 (124)  | 813 (96)   | 790 (106) |
|                  | 1.4 (1.0)  | 1.7 (1.5)  | 1.9 (1.7) |

Mean and standard deviation of reaction times (RT) and error rates (ER).

3.2. Manipulation check

3.2.1. iTBS blinding check

One patient in the sham group and three patients in the verum group did not respond when asked about perceived group allocation. In the verum group, 14/19 patients guessed their treatment condition correctly, as did 16/21 in the sham group. The proportion of correct guesses differed significantly from chance (0.5) in both groups (p = 0.027 for sham group, p = 0.031 for verum group).

3.2.2. Emotional Stroop task - behavioural data

For the clinical data, there was a significant main effect of the factor time in terms of a decrease of performance from baseline to post-iTBS regarding RTS (F1,42 = 4.622, p = 0.037) as well as ERs (F1,42 = 5.6, p = 0.007). Furthermore, a significant main effect for the factor condition (F1, 42 = 180, 109, p < 0.001) and the factor group (F2,42 = 2.42, p = 0.04) was detected for ERs only. As can be seen in Table 2, all subjects committed more errors for panic-related words than for neutral words but the sham-stimulated patients generally committed the fewest errors (verum vs. sham: t24 = 2.098, p = 0.047; controls vs. sham: t20 = 2.958, p = 0.006). There were no significant interactions. Mean RTS and ERs are for all groups, times and conditions are shown in Table 2.

3.3. fNIRS data - baseline differences and treatment effects

For the clinical data, 2 × 3 RM-ANOVA, CBSI concentrations revealed no significant main effects, but a significant three-way interaction of condition * time * group for both the left (F2,42 = 0.017, p = 0.023) and right PFC (F2,42 = 3.836, p = 0.027).

For the left ROI, separate post-hoc analyses for each time point displayed a significant difference in prefrontal activation for panic vs. neutral words for the two PD patients groups at baseline whereby the patients showed less prefrontal activation in response to panic than to neutral words (sham, panic vs. neutral: t20 = −2.643, p = 0.016; verum vs. neutral: t20 = −2.126, p = 0.047), but not at post-iTBS. No difference was found for the control group (Fig. 3a) at either time point.

Further post-hoc analyses of the changes of CBSI concentration over time (baseline vs. post-iTBS) in each group separately revealed a significant effect for the left PFC only in the verum group with a decrease in activation for neutral words (t19 = 2.220, p = 0.039) and an increase for panic-related words from baseline to post-iTBS (t19 = −2.454, p = 0.024) (Fig. 3b).

Comparing the three groups (verum, sham, controls) directly with each other, we further found a differentiation between the verum and the sham group for neutral words, whereby CBSI concentration was higher in the sham group (t19 = 2.208, p = 0.033). Concerning the right PFC, pairwise comparisons of activation for panic vs. neutral words showed no significant differences for any group at any measurement time. Similar to the results of the left PFC, there was a significant change from baseline to post-iTBS in the verum group, where the direction of change was the same as for the left PFC (increased activation for panic-related words: t19 = 3.062, p = 0.006, decreased activation for neutral words: t19 = 2.204, p = 0.040) (Fig. 3b).

Pairwise group comparisons showed significant differences in activation patterns only for post-iTBS with less activation for panic-related words (t19 = 2.052, p = 0.047) and more activation for neutral words (t19 = 2.528, p = 0.016) in the control group compared to the sham group.
verum group. The same pattern emerged when contrasting the sham and verum group: verum-stimulated patients showed more activation for panic-related words ($t_{39} = -2.054, p = 0.047$) and less activation for neutral words ($t_{39} = 2.420, p = 0.020$). There were no significant differences in CBSI concentration levels between sham and control group for either panic-related or neutral words at any measuring time.

Regarding the RM-ANOVA for the temporal control region, no significant effects were observed.

3.4. Clinical data

For the total scores (PAS-total, HAM-A total, CAQ-total), $2 \times 3$ RM-ANOVA revealed significant main effects for the factors time and group, as well as a significant time $\times$ group interaction (all $p \leq 0.001$). For both time points (baseline and post-iTBS), patients (verum and sham group) scored significantly higher on the clinical ratings than healthy controls. Post-hoc analyses further showed that patients’ scores (verum and sham) on HAM-A-total, observer- and self-rated PAS-total and CAQ-total decreased significantly from baseline to post-iTBS. However, no significant differences between the verum and sham group were found (please refer to Deppermann et al., 2014).

For the entire group of patients (verum and sham), scores of all subscales decreased significantly from baseline to follow-up 2 after 6 months, as shown in a significant main effect of the factor time (all $p < 0.05$, for further details please refer to the supplementary material). However, there were no significant differences between the sham and verum group. Additionally, a significant interaction of time and iTBS group was found for self-rated agoraphobic avoidance (Table 3). Post-hoc analyses revealed, under sham iTBS, a significant decrease from baseline to post-CBT, follow-up 1 and follow-up 2, but a significant increase of agoraphobic symptoms from follow-up 1 to follow-up 2. Verum iTBS resulted in significantly reduced self-rated avoidance behaviour for the comparisons baseline vs. post-CBT, vs. follow-up 1 and vs. follow-up 2. Also, agoraphobic symptoms declined significantly from post-iTBS to follow-up 1 and follow-up 2 (Table 3).

For the remaining subscales, no significant interactions of time and iTBS group were found.

3.5. Correlation of fNIRS patterns and clinical data

Considering changes over time (post-iTBS - baseline), no significant correlations were discerned for the verum or sham group.

4. Discussion

In this randomised, sham-controlled iTBS study, we set out to investigate via fNIRS whether (a) we could confirm prefrontal hypoactivation in PD patients (as compared to healthy controls) during an emotional regulation task (Emotional Stroop), and if (b) this hypoactivation could be normalised over a course of 15 sessions of iTBS over the left dorsolateral PFC as an add-on treatment to state-of-the-art CBT. Additionally, we assessed the impact of iTBS on clinical symptoms and evaluated whether changes in functional activation (as assessed via fNIRS) correlated with clinical change.

As expected, a significant left lateral prefrontal hypoactivation in response to panic-related, as compared to neutral, words could be detected in both patient groups, but not in the control group prior to the beginning of treatment. The effect was restricted to the left PFC. Hence, we were able to confirm a left-lateralized reduced prefrontal response to fear-related, compared to neutral, stimuli in PD patients which did not occur in healthy controls.

Over the course of the combined iTBS and CBT intervention, this baseline prefrontal hypoactivation of the left PFC disappeared for both the sham and the verum group, pointing to a general, beneficial effect of CBT which is in line with previous studies investigating the neurobiological effects of CBT (Clark and Beck, 2010). It further speaks in favour of the assumption that one mode of action of CBT is the modification of cognitive processes which are again related to prefrontal activation (Clark and Beck, 2010). Further, when comparing changes in CBSI concentration over the course of add-on iTBS, significant alterations were only found for the verum group, whereby prefrontal activation decreased for neutral words and increased for panic-related words. These results are in line with our assumption that iTBS can enhance prefrontal activity with respect to fear-relevant stimuli. Interestingly, these treatment effects were not only found for the left hemisphere, where the stimulation occurred, but also for the right PFC. Previous studies (e.g., Ilmoniemi et al., 1997) have also reported that rTMS may cause activation changes not only in the ipsilateral, but also the contralateral hemispheres. In contrast, the sham and control group did not show significant activation changes over time.

To rule out that the iTBS-effect for the verum group merely represented a more general measurement effect without task specificity, we tested the temporal fNIRS channels for similar alterations in CBSI concentration. However, no significant activation changes were revealed for this cortical non-ROI, supporting an interpretation in terms
Table 3
Clinical course of agoraphobic avoidance behaviour from baseline to follow-up 2.

| Measurement time | Verum (n = 22) | Sham (n = 22) | F(df, p) | Post hoc tests |
|------------------|----------------|---------------|---------|---------------|
|                  | Mean (SD)      | Mean (SD)     | Time    | Patient group - total | Verum group | Sham group |
|                  | F(df, p)       | Time × group  |         |                |             |            |
|                  | (n = 22)       |               |         |                |             |            |
|                  | Mean (SD)      | Mean (SD)     | Time    | ns.             |             |            |
|                  |                 |               |         | Baseline > Post-iTBS |             |            |
|                  |                 |               |         | Post-CBT***Follow-up 2*** |             |            |
| PAS (OR) Agoraphobic avoidance | Baseline       | 1.91 (1.22)   | 1.39 (1.19) | F<sub>6,252</sub> = 7.91, < 0.001 | Baseline > Post-iTBS | Post-CBT***Follow-up 2*** |
|                  | iTBS-7         | 1.35 (1.14)   | 0.96 (1.04) | | | |
|                  | iTBS-14        | 1.14 (1.23)   | 1.00 (1.03) | post-iTBS > Post-CBT | | |
|                  | post-iTBS      | 1.20 (1.08)   | 1.20 (1.24) | | | |
|                  | post-CBT       | 0.85 (1.04)   | 0.82 (0.99) | | | |
|                  | Follow-up 1    | 0.50 (0.77)   | 0.88 (0.93) | | | |
|                  | Follow-up 2    | 0.77 (0.92)   | 0.80 (1.10) | Follow-up 1 = Follow-up 2 | | |
|                  | iTBS-14        | 2.22 (1.01)   | 1.80 (1.10) | F<sub>4,179</sub> = 9.6, < 0.001 | Baseline > Post-CBT | Post-CBT***Follow-up 2*** |
|                  | post-iTBS      | 2.03 (1.02)   | 1.80 (1.10) | | | |
|                  | post-CBT       | 1.97 (0.87)   | 1.58 (1.04) | | | |
|                  | Follow-up 1    | 1.74 (0.70)   | 1.50 (1.14) | | | |
|                  | Follow-up 2    | 1.54 (0.82)   | 1.11 (0.98) | | Follow-up 1 < Follow-up 2 | |
|                  |                  | 1.18 (0.91)   | 1.08 (0.86) | | | |
|                  |                  | 1.29 (0.89)   | 1.35 (0.80) | | | |

CBT, cognitive behavioural therapy; df, degrees of freedom; F, F-value; ns., not significant; OR, observer-rated; p, p-value; PAS, Panic and Agoraphobia Scale; SD, standard deviation; SR, self-rated; Only significant ANOVA-results are depicted. P-values of ANOVA are Bonferroni-Holm corrected according to the topics described in the methods section.

* Significant at a significance level of p ≤ 0.05.

** Significant at a significance level of p ≤ 0.01.

*** Significant at a significance level of p ≤ 0.001.
of iTBS-induced prefrontal activation changes to fear-related stimuli. Interestingly, this conclusion, in terms of a fear-specific modulation of prefrontal activation patterns via iTBS, is also supported by the results of our cognitive paradigm we assessed within the same study. Here we observed general prefrontal hypoactivity which was, however, not affected by iTBS application (Deppermann et al., 2014).

While we found significant clinical improvement on all questionnaires, we could not find a general therapy-enhancing effect of iTBS in the verum group. Specifically, for the verum and sham groups, we found a significant improvement of clinical symptoms from the beginning of treatment interventions to the end of iTBS treatment. Also, during the complete time course of CBT, symptom severity measured on clinical total- and subscales further improved significantly. For the total scores of the clinical ratings, differences between the sham and verum group could not be found, neither after iTBS treatment nor at the end of CBT. However, the reduction of self-rated agoraphobic avoidance was more stable over time in the verum group. Notably, agoraphobic avoidance in the verum group decreased with some temporal delay after the last iTBS session. This might be due to the general effect of CBT including the exposure session. However, delayed onset of action has also been reported for rTMS for major depression (Schutter, 2009) and might thus be a characteristic of rTMS treatment. More studies with adequate follow-up assessments are needed to clarify this matter. The lack of a general therapy-enhancing effect of iTBS add-on treatment might be a ceiling effect. Alternatively, the timing of iTBS relative to CBT might have been suboptimal. We delivered iTBS during the first three weeks of CBT, which were dedicated to psychoeducation about PD. In contrast, the active parts of CBT (i.e., exposure sessions) took place after the administration of iTBS. iTBS might have a stronger clinical effect if administered at the same time as the emotional learning, considered central to CBT (Craske et al., 2014), is actually taking place.

Looking at correlations between CBSI concentrations and clinical data, we could not find an association between treatment efficacy and changes in prefrontal activation patterns.

All participants committed more errors for panic-related than for neutral words, indicating that the Stroop paradigm did induce emotional interference as intended, in line with Dresler et al. (2012). The fact that all participants showed this effect may be due to the panic-related words (e.g. death) being associated with negative emotions not only in patients but also in the control group. In fact, an Emotional Stroop effect for negative words has been reported for healthy subjects (e.g. Bar-Haim et al., 2007). Surprisingly, sham-stimulated patients generally committed the fewest errors, whereas no differences between the verum-stimulated patients and the control group could be found. This finding is hard to interpret, but it should be kept in mind that the behavioural data were only analysed for a smaller subsample, possibly causing some effects that are not representative for the whole sample. Generally, more errors were committed at the second measurement time accompanied with an increase in RTs pointing to a motivational decrease. The missing differences in RTs between controls and PD patients might also be due to the relatively small subsample. Another explanation, given by De Cort et al. (2008), might be that external stressors like the experimental set-up (which may also increase the general stress level in the control group) can explain a missing Stroop effect.

5. Limitations

Some considerations and limitations of this study should be discussed. As in the majority of clinical rTMS studies, the insufficient blinding certainly represents a limitation. However, only patients who received verum iTBS showed an increase of panic-specific cortical activation not only in the left, but also in the right, PFC. This could indicate a more pronounced, broader cortical activation, specifically induced by verum iTBS. For future studies, sham coils evoking scalp muscle stimulations should be used (e.g., Mennemeier et al., 2010). It should further be considered that other factors, like state-dependent neural baseline activity, might also have influenced iTBS effects.

For future iTBS studies, it might be interesting to investigate its potential therapeutic add-on effects by systematically manipulating the activation of fear-relevant networks preceding iTBS application, and the timing of iTBS relative to the phase and contents of concurrent CBT. In this context, an especially interesting attempt might be the application of iTBS in order to enhance extinction learning. In fact, Guhn et al. (2014) could show that activating rTMS over the medial PFC improved the extinction of a previously conditioned fear reaction in a group of healthy adults. Regarding clinical populations, not much research exist until now. Marin et al. (2014) discusses two studies (Ousch et al., 2009; Boggio et al., 2010) were rTMS was successfully applied for improved extinction processes in groups of patients suffering from post-traumatic stress disorder. However, the authors also emphasise that further systematic studies are needed before establishing rTMS as an add-on tool in clinical applications. At last it might have been interesting to perform an additional fNIRS measurement after the completion of CBT and not just after the first weeks when additional iTBS application took place. This way it would have been possible to further analyse the duration of iTBS effects on the one hand but also the general effects of CBT on a neurobiological level in more detail.

6. Conclusion

We were able to demonstrate prefrontal hypoactivity for panic-related stimuli in PD patients, which could be normalised by add-on iTBS. Clinical ratings significantly improved during iTBS/CBT. No significant differences were found between verum and sham iTBS, except for a more stable reduction of agoraphobic avoidance in the verum group. Thus, the therapeutic potential of a combination of iTBS and CBT requires further investigation in future studies that systematically manipulate the mental activity (e.g., fear-network activation) of patients during iTBS, as well as the timing of iTBS relative to CBT contents.

Conflict of interest

The authors declare that they have no conflict of interest.

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Contributors

VA, A-CE, AJF and PZ designed the study and wrote the study protocol. SD, NV, SS, FBH, SN, TD and IL recruited participants and analysed data. SD conducted fNIRS measurements. NV, SD and SS
conducted CBT. SD, NV, and JD wrote the manuscript. All authors have contributed to and approved of the final manuscript.

Appendix A. Supplementary data

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References

American Psychiatric Association. 2000. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). American Psychiatric Publishing, Washington, DC.

Bandelow, B., 1997. Panic and Agoraphobia Scale (PAS). Hogrefe & Huber, Seattle.

Bandelow, B., Seidler-Brandler, U., Becker, A., Wedekind, D., Rüther, E., 2007. Meta-analysis of randomized controlled comparisons of psychopharmacological and psychological treatments for anxiety disorders. World J. Biol. Psychiatry 8, 175–187. http://dx.doi.org/10.1080/1562297060110275.

Bandelow, B., Zohar, J., Hollander, E., Kasper, S., Möller, H.J., 2008. WFSBP task force on mood disorders, treatment and guidelines. World J. Biol. Psychiatry 9, 1562–1570. http://dx.doi.org/10.1080/15622970801997772.

Clark, D.A., Beck, A.T., 2010. Cognitive theory and therapy of anxiety and depression: a contemporary view. J. Cogn. Psychotherapy: An Int J. 24, 1–17. http://dx.doi.org/10.1080/15578883.2010.488323.

Craske, M.G., Treanor, M., Conway, C.C., Zbozinek, T., Vervliet, B., 2014. Maximizing the cognitive task and its modulation via sham-controlled rTMS. Biomed. Res. Int. 2014, 267801. http://dx.doi.org/10.1155/2014/267801.

Dresler, T., Gohn, A., Tupa, S.V., Ellis, A.C., Herrmann, M.J., Fallgatter, A.J., et al., 2013. Revise the revised? New dimensions of the neurometanatomical hypothesis of panic disorder. J. Neural Transm. 120, 3–29. http://dx.doi.org/10.1007/s00429-012-0800-8.

Ellis, A.C., Schneider, S., Dresler, T., Fallgatter, A.J., 2014. Application of functional near-infrared spectroscopy in psychiatry. NeuroImage 85, 478–488. http://dx.doi.org/10.1016/j.neuroimage.2013.03.067.

Ellert, G.H., Thompson, R.N., Zvolensky, M.J., Edwards, K., Frazer, N.L., Haddad, J.W., et al., 2008. The anxiety questionnaire: development and preliminary validity. Behav. Res. Ther. 38, 1039–1055.

Ferrari, M., Quaresima, V., 2012. A brief review on the history of human functional near-infrared spectroscopy (fNIRS) development and fields of application. NeuroImage 63, 921–935. http://dx.doi.org/10.1016/j.neuroimage.2012.03.049.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 1996. Structured clinical interview for DSM-IV Axis I disorders, clinician version (SCID-CV). American Psychiatric Press, Washington, D.C.

Guhn, A., Dresler, T., Andreatta, M., Müller, L.D., Hahn, T., Tupak, S.V., Polak, T., Deckert, J., Herrmann, M.J., 2014. Medial prefrontal cortex stimulation modulates the processing of conditioned fear. Front. Behav. Neurosci. 8. http://dx.doi.org/10.3389/fnbeh.2014.00095.

Hamilton, M., 1996. Hamilton Anxiety Scale (HAM-A). In: G.P.S. (Ed.), Internationale Skalen Für Psychiatrie. Beltz Test GmbH, Göttingen (Hampshire, A., Chamberlain, S.R., Monti, M. M.).

Herwig, U., Satrapi, P., Schneiders-Lecuna, C., 2003. Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation. Brain Topogr. 16, 95–99. http://dx.doi.org/10.1023/A:1021288413750.

Herrmann, M., 2003. Meta-analytic study of augmentative transcranial magnetic stimulation: a meta-analysis of randomized placebo-controlled trials. J. Clin. Psychiatry 64, 99–102. http://dx.doi.org/10.15202/jcp.12r07996.

Hirogaki, K., Ohno, M., Manno, M., Itoh, M., Kato, M., Yamashita, S., et al., 2007. Sham transcranial magnetic stimulation using electrical stimulation of the scalp over the left dorsolateral prefrontal cortex in double-blind sham-controlled trials. J. Clin. Psychiatry 78, e122–9. http://dx.doi.org/10.4088/JCP.12r07996.

Kaplan, H.S., Sadock, B.J., 2007. Comprehensive Textbook of Psychiatry, 8th ed. Mosby, St. Louis.

Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1993. The genetics of panic attacks and panic disorder. Arch. Gen. Psychiatry 50, 266–72. http://dx.doi.org/10.1001/archpsyc.1993.03130030056004.

Kendler, K.S., Prescott, C.A., 2006. Genetic epidemiology of panic attacks, panic disorder, and agoraphobia in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry 63, 415–24. http://dx.doi.org/10.1001/archpsyc.63.4.415.

Kendler, K.S., Prescott, C.A., 2006. The value of repetitive transcranial magnetic stimulation for the treatment of panic disorder? J. Affect. Disord. 94, 153–8. http://dx.doi.org/10.1016/j.jad.2005.08.028.

Kendler, K.S., Prescott, C.A., 2006. The value of repetitive transcranial magnetic stimulation for the treatment of panic disorder. J. Affect. Disord. 94, 153–8. http://dx.doi.org/10.1016/j.jad.2005.08.028.

Kendler, K.S., Prescott, C.A., 2006. The value of repetitive transcranial magnetic stimulation for the treatment of panic disorder? J. Affect. Disord. 94, 153–8. http://dx.doi.org/10.1016/j.jad.2005.08.028.
Tsuzuki, D., Jurcak, V., Singh, A.K., Okamoto, M., Watanabe, E., Dan, I., 2007. Virtual spatial registration of stand-alone fNIRS data to MNI space. NeuroImage 34, 1506–1518. http://dx.doi.org/10.1016/j.neuroimage.2006.10.043.

Tupak, S.V., Dresler, T., Badewien, M., Hahn, T., Ernst, L.H., Herrmann, M.J., et al., 2013. Inhibitory transcranial magnetic theta burst stimulation attenuates prefrontal cortex oxygenation. Hum. Brain Mapp. 34, 150–157. http://dx.doi.org/10.1002/hbm.21421.

Urry, H.L., van Reekum, C.M., Johnstone, T., Kalin, N.H., Thurow, M.E., Schaefer, H.S., et al., 2006. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. J. Neurosci. 26, 4415–4425. http://dx.doi.org/10.1523/JNEUROSCI.3215-05.2006.

Wassermann, E.M., Zimmermann, T., 2012. Transcranial magnetic brain stimulation: therapeutic promises and scientific gaps. Pharmacol. Ther. 133, 98–107. http://dx.doi.org/10.1016/j.pharmthera.2011.09.003.

Wittchen, H.U., Wunderlich, U., Gruschwitz, S., Zaudig, M., 1997. Strukturiertes Klinisches Interview Für DSM-IV: SKID I Interviewheft - Achse 1: Psychische Störungen. Hogrefe, Göttingen.

Wittchen, H.U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., et al., 2011. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur. Neuropsychopharmacol. 21, 655–679. http://dx.doi.org/10.1016/j.euroneuro.2011.07.018.

Zaubler, T.S., Katon, W., 1998. Panic disorder in the general medical setting. J. Psychosom. Res. 44, 25–42. http://dx.doi.org/10.1016/S0022-3999(97)00134-7.

Zhang, Y., Duan, L., Liao, M., Yang, P., Liu, J., Shan, B., et al., 2011. [MRI for brain structure and function in patients with first-episode panic disorder]. Zhong Nan Da Xue Xue Bao. Yi Xue Ban. J. Cent. South Univ. Med. Sci. 36, 1170–1175. http://dx.doi.org/10.3969/j.issn.1672-7347.2011.12.008.

Zwanzer, P., Minov, C., Ella, R., Schule, C., Baghai, T., Möller, H.J., et al., 2002. Transcranial magnetic stimulation for panic. Am. J. Psychiatry 159, 315–316.

Zwanzer, P., Fallgatter, A.J., Zavorotnyy, M., Padberg, F., 2009. Anxiolytic effects of transcranial magnetic stimulation—an alternative treatment option in anxiety disorders? J. Neural Transm. 116, 767–775. http://dx.doi.org/10.1007/s00702-008-0162-0.