Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective processing

Q1 Peter Zwanzger a,1, Christian Steinberg b,1, Maimu Alissa Rehbein b, Ann-Kathrin Bröckelmann b, Christian Dobel b, Maxim Zavorotnyya, Katharina Domschkea, Markus Junghöfer b,*

a Department of Psychiatry, Mood and Anxiety Disorders Research Unit, University Hospital Muenster, D-48149 Muenster, Germany
b Institute for Biomagnetism and Biosignalanalysis, University Hospital Muenster, D-48149 Muenster, Germany

The dorsolateral prefrontal cortex (dlPFC) has often been suggested as a key modulator of emotional stimulus appraisal and regulation. Therefore, in clinical trials, it is one of the most frequently targeted regions for non-invasive brain stimulation such as repetitive transcranial magnetic stimulation (rTMS). In spite of various encouraging reports that demonstrate beneficial effects of rTMS in anxiety disorders, psychophysiological studies exploring the underlying neural mechanisms are sparse. Here we investigated how inhibitory rTMS influences early affective processing when applied over the right dlPFC. Before and after rTMS or sham stimulation, subjects viewed faces with fearful or neutral expressions while whole-head magnetoencephalography (MEG) was recorded. Due to the disrupted functioning of the right dlPFC, visual processing in bilateral parietal, temporal, and occipital areas was amplified starting at around 90 ms after stimulus onset. Moreover, increased fear-specific activation was found in the right TPJ area in a time-interval between 110 and 170 ms. These neurophysiological effects were reflected in slowed reaction times for fearful, but not for neutral faces in a facial expression identification task. While there was no such effect on a gender discrimination control task. Our study confirms the specific and important role of the dlPFC in regulation of early emotional attention and encourages future clinical research to use minimal invasive methods such as transcranial magnetic (TMS) or direct current stimulation (tDCS).

© 2014 Published by Elsevier Inc.

Please cite this article as: Zwanzger, P., et al., Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective pro..., NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.07.003

http://dx.doi.org/10.1016/j.neuroimage.2014.07.003

1053-8119/© 2014 Published by Elsevier Inc.
subjects revealed a stronger orienting-response towards angry faces 
(d’Alfonso et al., 2000). van Honk et al. (2002) could show that low-
frequency rTMS over the right PFC reduced vigilant emotional 
responses to fearful faces. Repetitive TMS of the right dLPFC was consis-
tently associated with disrupted prefrontal–amygdala connectivity in 
another study, which fits to the proposed role of the PFC in the regula-
tion of mood and anxiety (Vanderhasselt et al., 2011).

While probably the majority of studies have used hemodynamic 
functional neuroimaging techniques to investigate mechanisms of top-
down emotion regulation, the temporal dynamics of those mechanisms 
are less well explored. It is generally accepted that certain regions in the 
PFC exert top-down influences on the processing of visual information 
(e.g. Bar et al., 2006) and neurophysiological studies show that these in-
fluences might already take effect within the first 100 ms (Rudrauf 
et al., 2008; Bayle and Taylor, 2010; Steinberg et al., 2013). In a very re-
cent TMS and electroencephalography (EEG) study, Mattavelli et al. 
(2013) reported that stimulation of the right medial PFC modulated 
the amplitude of early EEG components such as the P1 and N1. Given 
that the generators of these components are believed to be located in 
extrastriate occipito-temporal and parietal cortices as well as in parts 
of the fusiform gyrus (e.g. Di Russo et al., 2001), these results demon-
strate that the PFC is able to exert top-down influences on posterior 
and even distant brain areas in a rapid fashion. Studies on non-
clinically low- and high-anxious individuals show that differences in 
early visual processing (P1, N1, P2) of emotional information are more 
pronounced in high relative to low anxious subjects (e.g. Bar-Haim 
et al., 2005; Holmes et al., 2008) whereas those differences between 
groups become attenuated (Holmes et al., 2008, but see Rossignol 
et al., 2005) or are even absent (Walentowska and Wronka, 2012) at 
later stages of processing. Similar results have been observed in pa-
patients. For instance, there is evidence for abnormalities in early visual 
processing of fear-related material in specific phobia, already at the 
stage of the P1 (80–130 ms), the N170 (130–220 ms, see e.g. Kolassa 
and Miltner, 2006; Kolassa et al., 2007) and the early posterior negativ-
ity (EPN, 150–280 ms, Mühlberger et al., 2009; Wieser et al., 2010).

Considering that hemodynamic investigations of anxiety patients 
showed diminished PFC recruitment during emotion processing (e.g. 
Bishop et al., 2004; Shin et al., 2005), which is often interpreted as an in-
sufficient top-down control on a hyperactive amygdala, it appears 
tempting to manipulate activity in PFC areas to achieve changes in the 
neural affective network. Such theory based manipulations of cortical 
functions might be very promising for future approaches in treating 
psychiatric diseases like anxiety disorders. Although rTMS seems to 
have anxiety-reducing effects (see Zwanzger et al., 2009), so far only 
few studies have investigated its use for modulating the processing of 
anxiety-related information. Instead, rTMS has been largely deployed 
as a new treatment method for major depression and consequently, 
there is only sparse evidence for possible therapeutic effects in anxiety 
and anxiety disorders.

The study at hand aims at further elucidating the role of the PFC in 
the direct and indirect cognitive – emotional control of brain areas impli-
cated in anxiety-related processing. It investigates what consequences a 
targeted inhibition of dLPFC function using low-frequency rTMS has on 
the time-course of fear-related emotion processing measured by 
means of magnetoencephalography (MEG). Building on the assumption 
that the dLPFC exerts an inhibitory control over the emotion network, 
we expected amplified affective processing already during early pro-
cessing stages as a consequence of reduced dLPFC inhibition.

Materials & method

General paradigm

Participants

Forty healthy right-handed adults (23 women, mean age = 26.3 years, age range = 19–38) with normal or corrected to normal vi-
sion participated in the experiment. All provided written informed con-
sent and were paid for participation. The study protocol was approved 
in accordance with the Declaration of Helsinki. All participants con-
firmed to have no recorded history of neurological or psychiatric 
disorder.

Please cite this article as: Zwanzger, P., et al., Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective pro..., Neuroimage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.07.003
Photographs of 40 individuals (20 male, 20 female) were used in the present study, in which the individuals displayed either a fearful or a neutral facial expression. The 80 stimuli (40 fearful, 40 neutral faces) were cropped (including hair) using Adobe Photoshop®, were converted to grayscale, and were placed above a homogeneous gray background. Images were 10.5 cm height (297px @ 72dpi) and the nasion was aligned to the center of the screen (fixation cross) to prevent additional eye movements. All stimuli were taken from the Karolinska Directed Emotional Faces (KDEF) database (Lundqvist et al., 1998).

Transcranial magnetic stimulation

Transcranial magnetic stimulation was performed using a MagVenture MagPro X100 Option stimulator (Magventure, Farum, Denmark). A 70-mm figure-eight shaped coil was used to apply 1 Hz low-frequency repetitive transcranial magnetic stimulation (rTMS) for 30 min over the right dIPFC with a stimulation intensity of 120% related to the individual motor threshold. Individual resting motor threshold (RMT) was determined for the left abductor pollicis brevis muscle (APBM) and defined as the minimum stimulus intensity that produces a motor evoked potential in at least 50% of 10 trials. The position of the right dIPFC was marked 5 cm anterior in a parasagittal line to the scalp location for optimal APBM stimulation according to the standard protocol used in the majority of clinical trials investigating the benefits of prefrontal rTMS in therapeutic interventions. Herwig et al. (2001) revealed that, if applied with this standard setting, TMS over the left hemisphere predominately targets the dIPFC areas BA8 and BA9, in a few cases also the premotor cortex (BA6, frontal eye field). The motor cortex related TMS positioning should account for the known frontal petalia which is qualified by extended right-hemispheric frontal and especially dIPFC regions (Watkins et al., 2001). We are thus confident that the stimulation in the present study targeted the right-hemispheric BA8 and BA9.

For sham stimulation the figure-eight coil was oriented tangentially to the skull without touching the scalp. This one-wing 90 degree sham stimulation is unlikely to induce any measurable electrical currents within the underlying brain tissue (see Lisanby et al., 2001). It should be noted that we used a between-subject design also to account for possible differences in muscular or scalp sensations evoked by sham or real TMS stimulations. Since subjects had no previous rTMS experience and were informed that the motor-threshold TMS test several days before the study began targeted the right hemisphere, subjects in the sham group should not have been able to detect any conspicuity. The influence of corresponding sources of errors for between group comparisons should thus be minimal.

Behavioral tests

Subjects had to complete three tests which assessed possible behavioral effects of the rTMS intervention: Before and after MEG measurements, participants had to rate each of the 80 facial stimuli with respect to the perceived hedonic valence and emotional arousal on a 9-point rating scale by means of the Self-Assessment–Manikin scales (SAM rating) devised by Bradley and Lang (1994). The valence scale ranged from unpleasant (1) to pleasant (9) and the arousal scale from calm (1) to exiting (9). In a second and a third test, participants had to identify the gender (male vs. female) and the facial expression (fearful vs. neutral) of consecutively presented faces via button press in a speeded forced choice discrimination task. The order of tasks (gender/emotion) as well as the assignment of response keys (left/right) was randomized across subjects. Presentation of stimuli was fully randomized in all behavioral tests. Valence and arousal rating data from one subject had to be rejected because of technical problems. Thus, SAM ratings from 39 subjects were analyzed. Valence rating data from two further subjects were discarded because of extreme values (more than 3 times the interquartile range of the boxplot). All post-hoc tests were corrected for multiple comparisons using the Bonferroni–Holm procedure.

Magnetoecephalography (recording and data processing)

Visual evoked magnetic fields were measured using a high-density whole-head 275-channel MEG system (Omega 275, CTF, VSM Medtech Ltd.) with first-order axial SQUID gradiometers. Four MEG sensors were permanently disabled during data recording. Continuous MEG data in the frequency band between 0 and 150 Hz were recorded with a sampling rate of 600 Hz. Data preprocessing, artifact rejection and correction, averaging, statistics, and visualization were conducted with the Matlab-based EMEGS software (www.emegs.org; Peyk et al., 2011). Responses were down-sampled to 300 Hz and filtered offline between 0.01 and 148 Hz. To optimize the detection of early and transient brain responses in the higher frequency range, further low-pass filtering was not applied. Data was averaged from 200 ms before to 600 ms after stimulus onset and baseline-adjusted using a 150 ms pre-stimulus interval. The method for statistical control of artifacts in high-density EEG/MEG data was used for single trial data editing and artifact rejection (Junghöfer et al., 2000). This procedure (1) detects individual channel artifacts, (2) detects global artifacts, (3) replaces artifact-contaminated sensors with spline interpolation statistically weighted on the basis of all remaining sensors, and (4) computes the variance of the signal across trials to document the stability of the averaged waveform. The rejection of artifact-contaminated trials and sensor epochs relies on the calculation of statistical parameters for the absolute measured magnetic field amplitudes over time, their standard deviation over time, the maximum of their gradient over time (first temporal derivative), and the determination of boundaries for each of these three parameters. On average, 2.6 (SD: 0.8) out of 271 sensors (9.96%) were interpolated and 43.8 (SD: 17.6) out of 640 (6.6%) trials were rejected by the artifact rejection procedure. The number of interpolated sensors and the number of rejected trials did not differ across the experimental conditions.

After averaging, cortical sources of the event related magnetic fields were estimated using the L2-Minimum-Norm-Estimates method (L2-MNE; Hämäläinen and Ilmoniemi, 1994). The L2-MNE is an inverse modeling technique applied to reconstruct the topography of the primary current underlying the magnetic field distribution. It allows the estimation of distributed neural network activity without a priori assumptions regarding the location and/or number of current sources (Hauk, 2004). In addition, of all possible generator sources only those exclusively determined by the measured magnetic fields are considered. A spherical shell with evenly distributed 2 (azimuthal and polar direction, radial dipoles do not generate magnetic fields outside of a sphere) × 350 dipoles was used as source model. A source shell radius of 87% of the individually fitted head radius was chosen, roughly corresponding to the gray matter depth. Across, all participants and conditions, a Tikhonov regularization parameter k of 0.2 was applied. Topographies of source direction independent neural activities—the vector length of the estimated source activities at each position—were calculated for each individual participant, condition, and time point based on the averaged magnetic field distributions and the individual sensor positions for each participant and run.

Combining high-density MEG with advanced source localization methods, analysis was based on the neural generator activities available for every test source, time point, and participant. To explore valence effects independent of brain stimulation, estimated neural activation in the pre-TMS/pre-Sham phase was analyzed. Data acquired during this baseline session were submitted to a repeated-measures ANOVA including the within-subject factor Valence (fearful vs. neutral) and the between-subject factor Group (Sham vs. TMS).

Please cite this article as: Zwanzger, P., et al., Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective pro... NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.07.003
To assess effects of brain stimulation, differences between pre- and post-TMS/Sham phases were submitted to an ANOVA with identical factors. This baseline-adjusted analysis takes possible influences of preexisting variations into account and captures possible fluctuations of brain activity (e.g. habituation effects) throughout the course of the whole experiment in an accurate manner (see Steinberg et al., 2012).

Non-parametric cluster based permutation tests as suggested by Maris and Oostenveld (2007) were applied. To this end, Monte Carlo simulations of identical analyses based on 1000 random permutations of the complete data set of participants and experimental conditions were conducted. Based on first level statistical effects with a significance criterion of $p < .05$, spatiotemporal clusters of at least six neighboring test sources and at least 15 ms intervals were used for the cluster based statistics ($p < .05$).

Identified spatiotemporal clusters showing significant modulations of neural activations were further analyzed using post-hoc t-tests in order to specify the direction of effects.

Procedure

Prior to the start of the study, subjects were randomly assigned to one of two experimental groups, either the Sham or the TMS group (between-subject design). At the beginning, individual head shapes were digitized using a Polhemus 3Space® Fastrack to determine the head coordinate system for later calculation of spherical head models. Next, participants were sat in a dimly lit, sound attenuated and magnetically shielded chamber. To monitor the participant’s head position and movement within the MEG scanner, three landmark coils were attached to the two auditory canals and the nasion. Visual stimulation was provided via a mirror system passing the image onto a translucent projection screen. Viewing distance was 86 cm corresponding to 7° of visual angle given the size of the stimuli (10.5 cm in height). Subjects were instructed to passively view the stimulation. Online monitoring of recorded magnetic fields for the occurrence of alpha waves served as an index for fatigue. According to this, no subject had to be excluded from the study. In the pre-TMS MEG phase (overall 9 min), all faces were presented in randomized order for 600 ms with an inter-stimulus interval (ISI) of 1100 ± 300 ms. In total, 40 fearful and 40 neutral faces (stemming from 40 individuals) were repeated four times comprising 320 stimulus presentations. Following this, participants had to complete the SAM rating as well as the emotion and gender discrimination tasks. In the SAM rating all 80 stimuli were presented for 600 ms and immediately after offset the SAM scales appeared. Responses were made by button presses while moving a green cursor to the desired position along the scales. In the emotion and gender discrimination task, subjects were instructed to respond as fast as possible. Stimuli were presented for 600 ms and as soon as the subject responded, the next trial was presented after an ISI of 700 ± 200 ms. After a short break repetitive TMS was delivered at 1 Hz with a total of 1800 pulses over a period of 30 min above the right DIPFC. As mentioned above, sham TMS was by turning the coil 90° with one wing oriented to the skull. During real or sham stimulation participants sat in a comfortable chair. Once rTMS or sham stimulation finished, the second MEG session followed. The experimental design of this post-TMS MEG session was identical to the pre-TMS session, except differing randomizations of the stimuli. Finally, participants had to complete the same behavioral tasks as at the beginning of the experiment (different randomization). Avoiding confusion, the assignment of response keys was kept constant related to the pre-TMS phase.

In contrast to the rule of thumb that inhibitory TMS effects on motor cortex excitability last for as long as TMS was applied (e.g. Chen et al., 1997), there is some evidence that rTMS effects of inhibitory DIPFC stimulation might last for at least a longer duration. For instance, Eisenegger et al. (2008) applied 1 Hz low-frequency rTMS to the right DIPFC for 15 min and showed that an increase of the regional cerebral blood flow (rCBF) measured by means of positron emission tomography (PET) lasted for at least 9 min before returning to baseline after 13 min. Assuming a linear relationship, effects after 30 min rTMS could be expected to last for at least 18 min. MEG data, SAM ratings and emotion/gender ratings were acquired within 5–14, 16–19½, and 20±27 minute time-intervals after rTMS stimulation, respectively (see timeline in Fig. 1). Thus, while neural activity measured by MEG should be fully affected by the stimulation, behavioral performance could be influenced to a lesser degree. However, since neural measures were of predominant importance, MEG data were always acquired first. Overall, it took the participants roughly about 90 min to complete the whole experiment.

Results

MEG results

Baseline valence effects

In the baseline session prior to rTMS or Sham intervention, strong main effects of Valence were observed in an interval ranging from 60 to 160 ms with greater responses for fearful as compared to neutral faces. These affect-specific responses occurred in bilateral occipital and temporal areas (see Fig. 2). More specifically, differential activation was found in a bilateral occipital ($F(1,38) = 32.73; p < .001$), a left anterior temporal ($F(1,38) = 24.68; p < .001$) and a right mid-temporal cluster ($F(1,38) = 16.06; p < .001$). Fearful faces evoked stronger neural activation compared to neutral faces in all regions. There were no main effects of Group or interaction effects of Valence and Group. Thus, the two groups did not differ in overall brain activation and in emotional processing before the intervention.

Effects of rTMS

The spatiotemporal analysis of estimated neural activation revealed general and valence-specific effects of treatment significant on the cluster level in two intervals and two regions.

As shown in Fig. 3, a widely distributed source cluster covering the occipital, superior temporal, and parietal areas of the left hemisphere showed a huge main effect of Group in a time-interval ranging from 90 to 150 ms. To assess hemispheric specificity of this effect, source activity at two mirror symmetric source clusters on the left and right hemisphere within this interval were analyzed by a Valence (fearful vs. neutral) by Hemisphere (left vs. right) by Group (TMS vs. Sham) ANOVA.

This analysis revealed a main effect of Group which was characterized by a general increase of neural activity in the TMS as compared to the Sham group ($F(1,38) = 5.47; p = .025$). This main effect was modulated by a Valence × Group interaction ($F(1,38) = 4.44; p = .042$) as valence-specific difference activations (fearful vs. neutral) were relatively increased after TMS stimulation ($t(19) = 2.32; p = .031$), while emotion processing in the Sham group did not change ($t(19) = −0.78; p = .443$). There was also a significant Group by Hemisphere interaction ($F(1,38) = 4.71; p = .036$) indicating stronger activity in the right compared to the left hemisphere in the Sham group ($t(19) = 2.47; p = .035$) whereas as consequence of the predominately left hemispheric activation increase in the TMS group — no such effect was observed in the TMS ($t(19) = −0.86; p = .403$) group.

In another interval between 110 and 170 ms, group-dependent emotional modulation was found above right-hemispheric superior temporal and inferior parietal areas, presumably reflecting neural activity around the temporal parietal junction (TP), see Fig. 4. As above, laterality effects were assessed by comparing evoked neural activity in two mirror symmetric left and right-hemispheric clusters with a Valence by Hemisphere by Group ANOVA.

There was a main effect of Group ($F(1,38) = 6.32; p = .016$) again stemming from relatively increased activity in the TMS as compared to the Sham group. Furthermore, a significant three-way interaction of Valence, Hemisphere, and Group ($F(1,38) = 5.43; p < .025$) revealed that there was a substantially larger
right-hemispheric difference between fearful and neutral face processing in the TMS (t(19) = 2.17; p = .043) compared to the Sham group (t(19) = −1.69; p = .107), whereas no such difference occurred in the left hemisphere (all p > .05).

In summary, rTMS over right-hemispheric dlPFC led to an overall amplified face processing between 90 and 150 ms irrespective of stimulus valence in bilateral parietal, temporal, and occipital areas. In addition, the TMS group exhibited a treatment-related affect-specific boost in the processing of fearful vs. neutral faces which has not been observed in the Sham group. The same kind of effect, an additional increase of fear-specific activation, also appeared between 110 and 170 ms around the right TPJ area. The global power2 of the difference source waveforms (post–pre session) for the cluster in each hemisphere is shown in Fig. 5.

**Behavioral results**

**SAM ratings**

*Valence.* The repeated-measures ANOVA including the within-subject factors Session (pre-TMS vs. post-TMS) and Valence (fearful vs. neutral) and the between-subject factor Group (TMS vs. Sham) yielded a significant main effect of Session (F(1,35) = 4.972; p = .033) showing that arousal ratings decreased from pre-TMS/post-Sham (mean = 3.61; SD = 1.32) to post-TMS/post-Sham (mean = 3.34; SD = 1.39) sessions and that they were higher for fearful (mean = 4.68; SD = 1.79) as compared to neutral (mean = 2.27; SD = 1.08) faces. Again, there was neither a main effect of Group nor an interaction with other factors (all p > .05). The participant’s gender did not influence arousal ratings (all ps > .05).

*Emotion and gender discrimination task*

We measured accuracy (error rates) and reaction times (RTs) for each participant in the emotion and gender discrimination tasks. Data from three subjects had to be excluded from further analysis because of technical problems, and hence 37 subjects remained in the data set. Please note, when error rates and even RTs were compared within conditions, there were not any significant differences between groups for both the emotion and the gender tasks. Thus, differences in task difficulty are unlikely to account for the effects reported below.

In the emotion discrimination task, two outliers (more than 3 times the interquartile range of the boxplot) were detected and excluded from further analysis. Repeated-measures ANOVA featuring the factors Session (pre-TMS/pre-Sham vs. post-TMS/post-Sham), Facial Expression (fearful vs. neutral) and Group (TMS vs. Sham) revealed a main effect of Session (F(1,33) = 6.078; p = .019) characterized by a general decrease in reaction time between pre (mean = 828 ms; SD = 172 ms) and post (mean = 772 ms; SD = 146 ms) sessions. A main effect of Facial Expression (F(1,33) = 42.201; p = .000) showed that fearful faces (mean = 741 ms; SD = 146) were recognized much faster in comparison to neutral faces (mean = 859 ms; SD = 163). There was also a Session × Facial Expression interaction (F(1,33) = 10.457; p = .003), stemming from a decrease in RT for neutral faces (t(34) = 3.43; p = .002) from pre to post whereas RTs did not change for fearful faces (t(34) = 1.00; p = .322). In spite of the training effect for neutral faces, RTs for fearful faces (mean = 729 ms; SD = 133) were still significantly (t(34) = −4.83; p < .001) faster than for neutral faces (mean = 815 ms; SD = 174), even if only post session values were considered. Most importantly, these effects were modulated by an interaction of Session, Facial Expression, and Group (F(1,33) = 6.078; p = .019). Subjects in the TMS group were relatively slower in rating fearful than neutral faces after TMS intervention (t(15) = 3.33; p = .005), whereas...
subjects in the Sham group became generally faster in responding to both fearful and neutral faces while the difference between RTs for fearful and neutral faces ($t(18) = .675; p = .508$) was not significant (see Fig. 6). The gender of the participants did not influence RTs.

Regarding accuracy, after eliminating three outliers (more than 3 times the interquartile range of the boxplot), we found a significant Session × Facial Expression interaction ($F(1,32) = 12.992; p < .001$). Post-hoc t-tests revealed that error rates for fearful faces decreased from pre-TMS/pre-Sham (mean = 6.91; SD = 7.05) to post-TMS/post-Sham (mean = 3.6; SD = 4.53) session ($t(33) = 3.1; p = .004$) while errors for neutral faces from pre-TMS/pre-Sham (mean = 2.43; SD = 3.56) to post-TMS/post-Sham (mean = 3.6; SD = 4.23) sessions slightly increased ($t(33) = −2.136; p = .04$). No main effect or interactions with Group were found.

For the analysis of the RT data in the gender discrimination task three subjects had to be excluded from further analysis (more than 3 times the interquartile range of the boxplot). In the remaining data set we found a main effect of Session ($F(1,32) = 19.5; p < .001$) and a main effect of Face Gender ($F(1,32) = 42.851; p < .001$). Reaction times were faster in the post-TMS/post-ShAM (mean = 647 ms; SD = 90) as compared to the pre-TMS/pre-ShAM (mean = 684 ms; SD = 106) session and for recognizing male (mean = 630 ms; SD = 89.8) compared to female...
Examining accuracy in the gender discrimination task, a main effect of Face Gender was found ($F(1, 35) = 20.947; p < .001$). That is, subjects made less errors when seeing a male (mean = 5.54%; SD = 4.93) as compared to a female face (mean = 1.32%; SD = 1.79). No further significant effects were found.

It should be noted that the mean RTs calculated across sessions and conditions showed that both the TMS group (Emotion: mean = 767 ms, SD = 99 ms; Gender: mean = 654, SD = 64; $t(15) = 4.659$; $p = .000$) and the Sham group (Emotion: mean = 827 ms, SD = 174 ms; Gender: mean = 694, SD = 139; $t(18) = 8.16$; $p = .000$) were unexpectedly faster in discriminating gender compared to emotion. Although the corresponding error rates for the emotion rating (TMS: mean = 3.7%, SD = 2.5%; Sham: mean = 4.5%, SD = 3.4%; $t(32) = -.85; p = .401$) and the gender rating (TMS: mean = 3.5%, SD = 2.5%; Sham: mean = 3.4%, SD = 2.2%; $t(35) = .10; p = .915$) did not differ between groups, these overall RT effects could indicate some differences in task difficulty which would limit inferences regarding the affect specificity of the result.

However, training effects as reflected by RT reductions from pre to post measures for emotion and gender ratings did not differ between groups. Based on the RT difference values for emotion ($\Delta^{\text{Post-pre}}$ mean[feurful/neutral]) and gender ($\Delta^{\text{Post-pre}}$ mean[male/female]) ratings, both the TMS (Emotion: $\Delta^{\text{Post-pre}} = -42.6$ ms, SD = 99.2 ms; Gender: $\Delta^{\text{Post-pre}} = -35.5$ ms, SD = 46.2 ms; $t(15) = -3.43; p = .036$), and the Sham group (Emotion: $\Delta^{\text{Post-pre}} = -66.4$ ms, SD = 151 ms; Gender: $\Delta^{\text{Post-pre}} = -49.7$ ms, SD = 67.1 ms; $t(18) = -6.04; p = .553$) showed comparable training effects in the emotion and the gender ratings. These results suggest the absence of a floor effect in the gender condition. Thus, an effect of treatment on the gender rating could have been observed if present, which is why differences in task difficulty are rather unlikely to account for the reported results.

Discussion

The present study investigated the impact of inhibitory rTMS of the right dPFC on early affective processing as measured by differential MEG activation during the presentation of fearful and neutral faces. Our results showed that dPFC stimulation was followed by overall increased event related neural activity at bilateral parietal and temporal
cortex regions starting around 90 ms after stimulus onset. Importantly, rTMS-treatment of the right dlPFC led to not only a global increase of neural activity, but also stronger differential emotional responses to fearful compared to neutral faces in predominately right-hemispheric temporal visual areas from 110 up to 170 ms and additionally also in the left-hemispheric occipito-parietal regions from 90 to 150 ms. It should be mentioned that the analyzed time-intervals and regions were partially overlapping, and thus, it seems plausible that the interaction found in the early interval partially reflects the interaction found in the later time-interval. Behavioral tests revealed an adverse effect of rTMS on judging fearful facial expressions, but not on judging stimulus gender. Overall, the electrophysiological results suggest that diminished dlPFC functioning boosts preferential processing of emotionally relevant material in cortical affective networks. However, the behavioral data suggest that the dlPFC dysfunction eventually interferes with task-relevant behavior if proper affective processing is required to complete the task.

As described by Corbetta and Shulman (2002), activation of temporoparietal and inferior frontal areas may serve as a detection system for behaviorally relevant stimuli that is particularly sensitive to salient or unexpected cues. Therefore, it might be suggested that rTMS-induced changes in cortical network activity enhance specific attentional networks responsible for the detection of unexpected situations. Increased attention towards dangerous situations following rTMS-induced inhibition of right dorsolateral prefrontal activity is in line with several reports showing behavioral effects of inhibitory transcranial magnetic stimulation. According to work by van Honk and Schutter, low-frequency rTMS of the right dlPFC led to altered patterns of anxiety-related behavior (Schutter et al., 2001; van Honk et al., 2002). Similarly, in an experiment focusing on selective attention to angry faces, the same rTMS mode resulted in an increase of selective attention towards angry faces, the same rTMS mode resulted in an increase of selective attention towards angry faces (d’Alfonso et al., 2000).

Interestingly, such effects have also been observed in patients. Apart from a large body of evidence on antidepressant effects of rTMS over the dlPFC (Burt et al., 2002; Couturier, 2005; Gross et al., 2007; Martin et al., 2003; Slotema et al., 2010), several reports conclude on putative anxiolytic effects of rTMS which would render it an effective method for the treatment of anxiety disorders (Zwanzger et al., 2009). The majority of these studies were conducted in patients with posttraumatic stress disorder (PTSD). Here, clinical effects were observed in small case studies showing a decrease of PTSD symptomatology after right prefrontal treatment (Grissaru et al., 1998; McCann et al., 1998). Moreover, beneficial effects were reported in patients with panic disorder receiving suprathreshold low-frequency rTMS over the right prefrontal cortex (Garcia-Toro et al., 2002; Mantovani et al., 2007; Prasko et al., 2007; Zwanzger et al., 2002). Although the total number of studies is limited compared to the large set of data in major depression these results point towards anxiety-modulating effects of rTMS in anxiety and anxiety disorders and therefore underline the important role of the right dorsolateral prefrontal cortex in the processing of anxiety-related stimuli.

However, the majority of these studies have applied different rTMS protocols ranging from single-treatment sessions up to daily treatment for 2–3 weeks. Since some studies show that a single-treatment session of rTMS does not have any effect on anxiety (Zwanzger et al., 2009), it remains unclear, whether a single-treatment session is capable to modulate neuronal activity sufficiently to obtain clinically relevant effects. However, we argue that the effect size of the stimulation depends on the strength of the stimulus applied and we suggest that a lack of effects in experimental panic induction might be due to the strong stimulus of the panicogenic agent (cf. Zwanzger et al., 2009). Indeed, studies on attentional processing of emotional information reveal that a single session of rTMS can lead to significant effects. For instance, Leyman et al. (2005) showed that a single session of high-frequency rTMS, using a 10 Hz stimulation protocol over the right dlPFC, produced a significant impairment in the ability to inhibit negative information in a negative affective priming task. Similarly, in a double blind placebo-controlled crossover study, Vanderhasselt et al. (2009) reported that one session of high-frequency rTMS over the left dlPFC was followed by a significant improvement in task-switching performance, whereas no effect on mood was observed. Moreover, our findings fit nicely with previous reports showing opposite effects when high-frequency rTMS is applied over the right dlPFC. In a study using fMRI, De Raedt et al. (2010) showed that high-frequency rTMS of the right dlPFC resulted in an impaired disengagement from angry faces, which was associated with decreased activity in the right dorsolateral prefrontal and the cingulate cortex, but with increased activity in the amygdala. These results point towards an altered attentional control of affective processing following right prefrontal stimulation.

Our results might also be discussed in the context of hemispheric lateralization. According to the so called “valence-asymmetry hypothesis” after which withdrawal-related emotions such as anxiety are predominately processed by the right hemisphere, whereas the left hemisphere is more associated with approach-related emotions, such as positive mood (Davidson and Irwin, 1999). Along with this hypothesis, there is evidence that anxiety is associated with increased right-hemispheric activity (Heller et al., 1998). In light of this model, our strategy using inhibitory rTMS to decrease right dlPFC top-down emotional control might be interpreted as an approach of generally enhancing the right-hemispheric processing of fear relevant stimuli which at the same time is accompanied by increased selective attention towards threat. Alternatively, our results could also be explained by findings from face perception studies (e.g. Bentin et al., 1996; Kanwisher et al., 1997; Kloth et al., 2006) showing that there is a right-hemispheric dominance for processing faces which can be seen for facial emotional expressions as well (e.g. Pourtois et al., 2005). Thus, this dominance could have been lost as consequence of rTMS.

Finally, the results obtained in the current study accord with findings from basic and clinical affective research. For instance, ERP components in early and midlatency time-intervals such as the occipital P1 (90–130 ms) and the occipito-temporal early posterior negativity (EPN, 120–280 ms) have been found to be susceptible to different kinds of visual emotional stimuli. The P1 emotion effect is well documented for emotional facial expressions (Batty and Taylor, 2003; Dolan et al., 2006; Pizzagalli et al., 1999; Pourtois et al., 2005) and is believed to reflect the early encoding of emotional features and/or to be related to the (later) allocation of attentional resources to the corresponding stimuli. The EPN emotion effect has been found in response to various visual emotional stimuli such as faces (Schupp et al., 2004), scenes (Junghöfer et al., 2001), words (Kissler et al., 2007), and even hand gestures (Fiasch et al., 2009). This affect-specific response has been associated with facilitated emotional processing in ventral visual areas and has been interpreted in line with the concept of motivated attention (Lang et al., 1997). Research investigating the impact of clinical and subclinical anxiety on emotional processing showed that there are noticeable differences between individuals in the processing of affective stimuli depending on their actual anxiety level. These differences were found to mainly manifest in early and midlatency ERP components such as the already mentioned P1 (Kolassa and Miltner, 2006) and the EPN (Mühlerberger et al., 2009; Wieser et al., 2010). In the current study, the early and the midlatency activations seen in occipito-temporal areas are in line with previously described time-intervals and locations of the P1 and the (early phase of the) EPN components. According to the literature, the effects shown here may reflect the exaggerated perceptual processing of emotionally and motivationally significant cues which is associated with an increase of attention towards those stimuli.

Inhibitory rTMS of the right dlPFC led to a general amplification of brain activity in bilateral occipito-temporal–parietal cortex areas and

Please cite this article as: Zwanzger, P., et al., Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective pro..., NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.07.003
to increased affect-specific responses at the right TPI region. Inactivation of the right dlPFC might result in a loss of inhibition which is usually imposed on limbic structures and other cortical areas. In other words, releasing the ‘brake’ in the right dlPFC seems to influence affective visual processing at early stages and recalls how affective stimuli are processed in anxious people or individuals suffering from anxiety disorders. Our findings are important for not only understanding affective processing per se, but also might be of interest for future, more clinically orientated studies. For instance, in PTSD, a loss of (cortical) inhibition (and a hyperactivation of the amygdala) is discussed that might be compensatory by applying rTMS to the corresponding cortical regions. However, regarding the role of the dlPFC in affective processing and its contribution to anxiety disorders, more research is needed investigating e.g. the impact of dlPFC rTMS on the processing of pleasant emotions and the interplay between inhibitory/excitatory stimulation and left/right hemisphere.

Neurophysiological effects in this study come along with modulated behavior in an affect-specific discrimination, but not in an emotion-independent control task. Interestingly, the behavioral effects occurred after only one treatment session with rTMS and in spite of a relatively long time lag between brain stimulation and behavioral testing, which possibly reduced the impact of the treatment (see procedure above).

The emotion-selective deceleration of reaction times seen here corresponds with other reports showing interfered visual emotional face processing after TMS intervention. For instance, a significant slowing of reaction times has been reported for fearful compared to happy faces following TMS over right somatosensory cortex (Pourtois et al., 2004). In another study, TMS applied to the medial prefrontal cortex also led to prolonged reaction times, when angry facial expressions were presented to the subjects (Harmer et al., 2001). Of course, TMS stimulation sites vary across studies (including the present one), however, all targeted regions are relevant for the processing of emotionally relevant visual stimuli. The prolonged reaction times for identifying fearful faces in the current study might be explained in different ways: Inhibitory top-down control usually imposed by the dlPFC on emotion-related areas such as medial and ventrolateral PFC is important to appraise the relevance of a stimulus in a given context (e.g. Wessing et al., 2013) and thus prevents the occurrence of disproportionate emotional reactions towards affective stimuli. The TMS stimulation applied here might have caused a loss of control over emotional responses towards fearful faces, which amplified fear-specific reactions, such as fight or flight preparation, during affective stimulus presentations, and drew attentional resources away from the actual cognitive task (i.e., emotion categorization). TMS might have also disturbed the process of emotion categorizing per se which is why the fearful facial emotional expressions were more difficult to detect. Indeed, stimulus categorization is a function which is known to be mediated by the dlPFC (Cacioppo et al., 1993; Freedman et al., 2003). Thus, the impaired control of attention towards and/or the deteriorated categorization of emotional stimuli might account for the behavioral results seen in the current study.

Lastly, it should also be mentioned that there were not any effects of rTMS on affective face processing at the site of stimulation, at least for the analyzed time-intervals. This is in line with findings from other studies which did not report rTMS effects at site of stimulation (dlPFC), either, when regional cerebral blood flow or glucose metabolism was measured (Kimbrell et al., 2002; Oishiishi et al., 2004; Speer et al., 2003). However, based on the idea that rTMS might have evolved sustained effects which could not have overcome the cluster-level criterion, we specifically analyzed the complete time-interval (from 0 to 600 ms) for Group × Session and Group × Session × Valence interactions. As a result, one region at the site of stimulation as well as another corresponding contralateral region revealed an enhanced emotion-unspecific neural activity after rTMS but not after sham stimulation (please see supplementary content) which could be interpreted as an effect of valence-unspecific neural noise induced by rTMS (e.g. Walsh and Cowey, 2000). It remains unclear under which conditions one sided rTMS to the dlPFC evokes enhanced ipsilateral (Eisenegger et al., 2008) or enhanced bilateral (Nahas et al., 2001) neural activity at the site of stimulation.

Taken together, our data show that inhibitory rTMS of the right dlPFC is followed by an increased activation in response to potentially threatening stimuli in areas related to both visual (emotional) processing and visual selective attention. Overall the data confirm a specific and important role of the prefrontal cortex in the regulation of emotional attention and furthermore underline the idea of the dlPFC being a target for future clinical research and therapeutic intervention using minimally invasive methods such as transcranial magnetic (TMS) or direct current stimulation (TDCS).

Acknowledgments
This work was supported by a grant of the Deutsche Forschungsgemeinschaft SFB TRR-58 subproject C01 to Markus Junghöfer, Peter Zwanzger and Christo Pantev. We thank A. Wollbrink, Karin Berning, Ute Trompetter, Hildegard Deitermann and Janna von Beschwert for technical assistance. We also thank two anonymous reviewers for their helpful comments and suggestions to improve the quality of this work: The first two authors, Peter Zwanzger and Christian Steinberg, contributed equally to this work.

Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.07.003.

References
Mantovani, A., Lisanby, S.H., Pieraccini, F., Ulivelli, M., Castrogiovanni, P., Rossi, S., cerebral measurement of the induced electrical

Med. 39 (6), 1019
cortex on the inhibition of emotional information in healthy volunteers. Psychol.

tal cortex and artificial emotional faces differs between lower and higher socially anxious persons. J. Neurotransm. 116, 735–746.


From the top to the bottom, the text is clearly separated into paragraphs, each containing relevant research findings and studies related to the topics of neurophysiology, cognitive neuroscience, and transcranial magnetic stimulation (TMS). The text covers the following aspects:

- Effects of TMS on emotional processing and cognitive functions
- Differences in emotional processing between healthy and anxious individuals
- The role of the prefrontal cortex in emotional regulation
- The impact of TMS on regional cerebral glucose metabolism
- The influence of TMS on visual processing of emotional faces

The text is rich in scientific references and citations, indicating a thorough review of the literature. The findings are supported by empirical data from various studies, highlighting the multidisciplinary nature of the research field.

Vanderhasselt, M.A., Baeken, C., Hendricks, M., De Raedt, R., 2011. The effects of high frequency rTMS on negative attentional bias are influenced by baseline state anxiety. Neuropsychologia 49 (7), 1824–1830.


Please cite this article as: Zwanzger, P., et al., Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective processing in panic disorder. NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.07.003