Intermittent theta burst stimulation (iTBS) in the treatment of anxiety disorders – From neurobiological underpinnings to clinical applications

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…meine beiden Betreuer **Prof. Hautzinger und Prof. Fallgatter**, die es mir als quereinsteigende Medienwissenschaftlerin ermöglicht haben an der Klinik und der Fakultät anzufangen. Vielen Dank für diese Chance! Ich hoffe ich kann u.a. hiermit zeigen, dass ich sie genutzt habe.

… meiner Arbeitsgruppe **Psychophysiologie und Optischen Bildgebung**. Ihr seid meine Tübinger Ersatzfamilie und auch wenn ich öfters mal die Rolle des wütenden Kindes oder alternativ der grummeligen Großtante eingenommen habe, hab ich euch doch alle trotzdem lieb und habe viel von euch gelernt. Und ihr hoffentlich auch von mir ;-)

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Zusammenfassung

Die vorliegende Arbeit hatte es zum Ziel zu untersuchen inwiefern die intermittierende Theta Burst Stimulation (iTBS) eine wirksame Therapiemöglichkeit in der Behandkung von Angststörungen darstellen könnte. Der theoretische Hintergrund dieser Fragestellung basierte dabei auf der Annahme eines in der Literatur häufig berichteten Ungleichgewichts des *Angstnetzwerks*, welches sich durch präfrontale Hypoaktivität sowie Hyperaktivität subkortikaler Strukturen wie beispielsweise der Amygdala auszeichnet. Darüberhinaus wurde die Fähigkeit der repetitiven transkraniellen Magnetstimulation (rTMS), wie in diese Falle der iTBS, umschriebene kortikale Aktivierungsmuster auf eine nicht-invasive Art und Weise zu modulieren in mehreren Studien sowie klinischen Fallberichten gezeigt. Aus diesen Befunden abgeleitet wurden zwei Studien konzipiert, wobei beide eine jeweils unterschiedliche potenzielle Anwendung der iTBS zur Behandlung von Angststörungen untersuchen sollte. Folglich beschäftigte sich die erste Studie mit der Wirkung einer wiederholten (plazebo-kontrollierten) iTBS Applikation als zusätzliche Unterstützung während dem Verlauf einer manualbasierten kognitiven Verhaltenstherapie (15 Sitzungen während der ersten drei Wochen) in einer Gruppe von Patienten mit Panikstörung mit und ohne Agoraphobie. Die zweite Studie wiederum konzentrierte sich auf den Einfluss einer einmaligen iTBS Anwendung vor einer angstauslösenden Situation auf die Symptome in einer Gruppe von Spinnenphobikern (subjektiv wahrgenommene Angst sowie Verhaltens- und psychophysiologische Korrelate). In beiden Studien wurde die präfrontale Aktivität sowohl vor als auch nach der iTBS Behandlung mit Hilfe der Nahinfrarotspektroskopie aufgezeichnet und schließlich mit den Aktivierungsmustern einer gesunden Kontrollstichprobe verglichen.

Grundsätzlich konnten beide Studien Veränderungen im *Angstnetzwerk* hinsichtlich abweichender präfrontaler Aktivierungsmuster im Vergleich zur gesunden Kontrollgruppe replizieren. Weiterhin konnte gezeigt werden, dass diese Veränderungen sich nach iTBS Applikation teilweise normalisieren liesen. Eine klinische Verbesserung bezüglich einer subjektiv stärker ausgeprägte Symptomreduktion nach iTBS Anwendung konnte jedoch in keiner der beiden

Studien nachgewiesen werden. Mögliche Gründe und Schlussfolgerungen für zukünftige Studien zur klinischen Anwendung von iTBS werden diskutiert.

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“They tell us that the only thing we have to fear is fear itself, but I don't believe that." he said.

Then, a moment later, he added: "Oh, the fear is there, all right. It comes to us in many different forms, at different times, and overwhelms us. But the most frightening thing we can do at such times is to turn our backs on it, to close our eyes. For then we take the most precious thing inside us and surrender it to something else.”

Haruki Murakami,
Kafka on the shore

1. General Introduction

1.1 Outline of the present work

The overall goal of this dissertation was to further clarify the neuro-physiological characteristics of pathological anxiety and related cognitive and emotional biases in order to assess the potential of repetitive transcranial magnetic stimulation (rTMS) as a supportive tool in its treatment. To do so, two studies were conducted, whereby the first one addressed the question by exploring the prefrontal activation patterns of patients with panic disorder with and without agoraphobia prior to as well as after 15 (sham-controlled) rTMS applications over the left dorsolateral prefrontal cortex (DLPFC). In doing so, the rTMS treatment was performed as an add-on to cognitive behavioural therapy (CBT) which was conducted in a manual-based group setting. The outcome of this study resulted in two manuscripts, whereby the first one (section 2.1) focused on neurobiological alterations and their modulation via rTMS during a cognitive task in this group of patients. Respectively, the second one (section 2.2) further explored neuronal alterations during the processing of emotional stimuli as well as the overall improvement of clinical symptoms during the time course of CBT.

The second study was subsequently designed to examine the impact of prefrontal rTMS on emotional processing and emotion regulation in a more exclusive manner by specifically looking at a single (sham-controlled) rTMS session which was combined with a virtual reality (VR) challenge as a fear-inducing situation in a group of spider phobic participants. Again, two manuscripts emerged from this study. The first one (section 3.1) described the effects of the elicited fear during VR immersion on an electrophysiological level (heart rate, heart rate variability and skin conductance) as well as on a subjective level (anxiety and disgust ratings) in more detail. The second one (section 3.2) once again focused on prefrontal activation patterns before and after the rTMS-VR combination but this time with a special emphasis on the functional connectivity between different cortical areas. Moreover, changes in perceived valence and arousal ratings of the presented stimuli were reported. Preceding these manuscripts, the following section shall give a concise overview of the fundamental background in anxiety research which was taken as a basis for the

scientific deduction of the study designs. Hereby, the neurobiological findings which have been related to pathological anxiety as well as its state of the art treatment options as the foundation for further research are considered.

1.2 Pathological anxiety – global overview

In general, as a basic emotion, fear clearly has a functional adaptive value (Ekman, 1999). In this regard it initiates the well-known "fight or flight response" (Cannon, 1915) by activating the sympathetic nervous system, thereby enabling organisms to quickly react towards environmental stimuli which may threaten survival (Bracha, 2004). In this context it may also be argued that it further has a social function as the fear reaction of one individual can serve others as a warning sign of potential danger (Marsh, Ambady, & Kleck, 2005). Usually, the degree of danger should determine the intensity of the fear reaction thereby also modulating the behavioural response. However, depending on the individual's predisposition as well as experiences the responsiveness of the "fear circuit" (for a more detailed explanation please refer to the next section) may be increased, leading to hypervigilance as well as exaggerated cognitive and behavioural reactions to environmental stimuli (Rosen & Schulkin, 1998). Pathological anxiety can hence be defined as an oversensitive fear network which leads to an overestimation of the actual danger and manifests itself in situationally inadequate thoughts and actions. The negative thinking thereby typically includes all cognitive modalities such as attention, memory and judgement (Beck, Emery, & Greenberg, 2005). Behavioural reactions associated with pathological anxiety are mostly escape from or avoidance of the feared state or stimulus (Woody & Teachman, 2000).

In principle, reactions to threat can be described as an interplay between bottom-up and top-down processes (Kim et al., 2011). In this regard, bottom-up processing is necessary in order to respond fast and automatically to survival relevant stimuli while top-down processes are needed to include further knowledge or contextual information in order to regulate the emotion so the behaviour can be adapted.

Accordingly, pathological anxiety can be conceptualised as an impaired interaction of bottom-up and top-town processing. In this context, a specific phobia as for example spider phobia can be seen as a model for the development of anxiety disorders where the interplay of bottom-up and top-down processing is out of balance. In this case, the spider as the initially threatening stimulus activates a fear reaction via bottom-up processing. Whereas individuals without spider phobia are able to integrate further information about the spider (like that it is not harmful) and hence down regulate their emotion, a spider phobic is flooded by his initial fear. This may either be caused by a hyper vigilant bottom-up system or because the top-down regulatory system is not efficient enough, whereby, of course, both systems affect each other.

1.3 Neurobiological aspects of the fear response

1.3.1 The fear network: the influence of cognitive control on fear-inducing stimuli

The neurobiological correlates of the fear response have been addressed in a large number of studies and review articles (for example, Dresler et al., 2013; Gorman, Kent, Sullivan, & Coplan, 2000; Öhman & Mineka, 2001). In this regard, there is consensus that the amygdala may be seen as a core structure of fear processing which automatically gets activated by environmental as well as visceral stimuli. Via different nuclei it thereby receives input from a huge number of different brain areas, especially from primary sensory cortices as well as directly via the sensory thalamus. Contextual information is included through projections from the hippocampus. In turn, the central nucleus of the amygdala activates targets in the brainstem (as the locus ceruleus or the periaqueductal gray region) and hypothalamus which initiate the fear response by activation of the sympathetic nervous system as well as hypothalamic–pituitary–adrenocortical (HPA) axis and associated neurochemical reactions (Deppermann, Storchak, Fallgatter, & Ehlis, 2014). However, as already stated, an emotional reaction is not solely determined by the stimulus per se, but rather the integration of the stimulus within its context by including top-down

information. On a neurobiological basis, this is thought to be accomplished via reciprocal connections of the amygdala and the prefrontal cortex (PFC). In this regard, the medial PFC (MPFC) (Kim et al., 2011), which is again interconnected with other prefrontal areas such as the DLPFC as well as the anterior cingulate cortex (ACC), plays a crucial role in effective emotion regulation.

When speaking about emotion regulation, neuroimaging studies generally differentiate between two major strategies to influence the emotional response: attentional control and cognitive change (Ochsner & Gross, 2005). Hereby attentional control can be described as selectively focusing on either perceptual or emotional features of a stimulus or simply distracting yourself by thinking of something else thereby suppressing the emotional response. Cognitive change on the other hand includes anticipatory processes in terms of expectancies for pleasant or aversive experiences. Furthermore, cognitive change also comprises cognitive reappraisal as a strategy to actively influence the perception of a given stimulus by changing the interpretation of its meaning. Additionally to the medial and lateral PFC as well as the ACC, the insular and the orbitofrontal cortex (OFC) have been shown to be activated during these processes (Ochsner & Gross, 2005). Furthermore, a number of studies have shown that the inferior frontal gyrus (IFG), comprising parts of Broca´s area in the left hemisphere, is not only involved in language processing (Friederici, 2011) but also attentional processes (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010), emotion regulation (Goldin, McRae, Ramel, & Gross, 2008) and behavioural control (Swick, Ashley, & Turken, 2008).

All in all, it is important to note that prefrontal areas are not only involved in controlling or diminishing the emotional response, but also in generating or amplifying it (Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008), and they are also influenced by the input they receive from the amygdala (Gorman et al., 2000). In effect, the already described imbalance between bottom-up and top-down processing as a model for the expression of pathological anxiety could also repeatedly be shown on a neurobiological level whereby reciprocal inhibitory connections between PFC and amygdala may lead to hyperactivity of the amygdala on the one hand and prefrontal hypoactivation on the other hand (De Carvalho et al., 2010; Engel, Bandelow, Gruber, & Wedekind, 2009; Kent & Rauch, 2003; Nishimura

et al., 2007; Ohta et al., 2008). However, even though the common neurobiological model for pathological anxiety includes hypoactivation of prefrontal areas, it is important to note that there are also diverging findings. An example for this discrepancy in results may be given by the frequently applied Emotional Stroop paradigm which is generally assumed to assess emotional regulation by means of attentional processes (for example Todd, Cunningham, Anderson, & Thompson, 2012). The general idea behind this task is that reading is a rather automated process in adults, thus the meaning of a presented word will capture attention no matter whether it is relevant for the ongoing task thereby imparing behavioural performance. Further, negative compared to neutral word valence should lead to an even more distracting effect. This way, presenting disorder-specific stimuli should have a bigger impact within a particular group of patients when being compared to healty controls (Williams, Mathews, & MacLeod, 1996). However, when applying such a task, on the one hand, prefrontal hypoactivation due to inhibitory effects of a hyperactive amydgala in anxiety disorders may be expected. On the other hand however, it can also be assumed that a more negative valence of the presented words will capure more attention which will in turn lead to an increase in PFC activation in patients with anxiety disorders. In fact, both, prefrontal hypoactivation as well as prefrontal hyperactivation in response to fear-relevant words has been reported before (Chechko et al., 2013; Dresler et al., 2012; Puetz et al., 2016; Schienle, Schäfer, Walter, Stark, & Vaitl, 2005; Straube, Mentzel, Glauer, & Miltner, 2004; Tupak, Reif, et al., 2013). Thus, it is important to not look at behavioural and activational results separately but rather try and integrate both findings. Surely, further explanations for the discrepancy regarding the findings in terms of prefrontal hypoactivation versus prefrontal hyperactivation in anxiety disorders might simply be given by general differences of the applied tasks but possibly also by the use of the particular emotion regulation strategy. As a final remark in this context, it needs to be mentioned that, in line with the diverging findings on prefrontal activation, a very recent meta-analysis (Sobanski & Wagner, 2017) on the functional neuroanatomy of panic disorder also found that the presumed amygdala hyperactivation in anxiety disorders strongly depends on a number of factors including the presented stimuli or the experimental design but also the particular study population. Thus, this finding

again underlines the importance of not just considering the mere activatinal patterns but also the contextual circumstances of their appearance.

1.3.2 The valence hypothesis

For a long time, models of emotion processing have proposed that there is a hemispheric lateralisation regarding the valence of emotional stimuli. In this regard, positive or approach-related emotions are supposed to rather be processed in the left hemisphere while negative or withdrawal-related emotions are rather processed in the right hemisphere (Reuter-Lorenz & Davidson, 1981; Wedding & Stalans, 1985). In 1998, this hypothesis could for the first time also be validated by a neuroimaging study (Canli, Desmond, Zhao, Glover, & Gabrieli, 1998). Since then, a number of studies have replicated these findings (for example, Balconi & Mazza, 2010; Schutter, de Weijer, Meuwese, Morgan, & van Honk, 2008). As a consequence, one could hypothesise that individuals suffering from a psychological disorder which is associated with an increase in negative affect, such as major depression or anxiety disorders, should probably be characterised by a hemispheric disparity in terms of left lateralised hypoactivation and right lateralised hyperactivation. Current research could show that there is indeed evidence for this assumption. In this respect, studies with depressed patients repeatedly found left frontal hypoactivation or right prefrontal hyperactivation (Davidson, 2002; Henriques & Davidson, 1991, 2000). So far, findings are less clear for anxiety disorders, but there are hints that a similar hemispheric imbalance exists (Davidson, 2002; Wiedemann et al., 1999)) which would be in line with the idea of an altered network in pathological anxiety that is characterised by diminished prefrontal activation as described above. Within the framework of this idea, it may be assumed that the alterations of the fear network cannot only be detected when a fear-inducing stimulus is present, but can more generally be found across situations, e.g. also during the completion of cognitively demanding tasks which require the recruitment of prefrontal control (Ohta et al., 2008).

In fact, from animal as well as epidemiological and twin studies in humans, it may be assumed that the proness to experience increased state anxiety across different situations is associated with a generally increased sensitivity towards potentially fear-inducing stimuli. Thus, independent of a manisfest anxiety disorder, there seem to be certain relatively stable personality traits as for example harm avoidance which may in turn also be partly associated with the described alterations within the fear network (Kampman, Viikki, & Leinonen, 2017). Altogether, these changes within the fear network can presumably be accounted for by neurochemical processes. In this regard, a number of studies mainly found alterations in the gamma-aminobutyric acidergic (GABA) and serotoninergic neurotransmitter system (Dresler et al., 2012). Moreover, it is also likely that the endocannabinoid system indirectly plays a crucial role in modulating the neural activation during a fear response by disinhibiting prefrontal output neurons as well as influencing GABA release in the hippocampus (Deppermann et al., 2014).

1.3.3 Influence on heart rate and electrodermal activity

As the fear response includes the activation of the sympathetic nervous system, this leads to a number of homeostatic changes in the body which prepare the organism for action. In doing so, the preganglionic nerve fibres that innervate the adrenal medulla release acetycholine which in turn triggers the release of adrenaline as well as noradrenalin that finally acts on the cardiovascular system by increasing the heart rate (HR) and dilating the bronchi (Deppermann et al., 2014). Moreover, the blood pressure changes due to vasoconstriction, which supports the blood supply to those organs that are most important for the "fight or flight" reaction. At last, the sympathetic nervous systems also controls the activation of the sweat glands in the body whereby the sweat production increases during acute fear in order to cool the body down during the expected action (Drummond & Lance, 1987). This increase in sweat production is further associated with an increase in skin conductance which can be used to measure the electrodermal activity (EDA). Since especially HR and EDA are relatively easy to assess, a number of studies have been conducted which

showed that the mere presentation of pictures of potentially fear-relevant stimuli can cause changes in HR and EDA (Flykt, 2005). Going beyond the presentation of just two-dimensional visual stimuli, VR scenarios use specialised computer displays or headsets (head mounted displays, HMD) which simulate three-dimensional virtual rooms in which the user is able to interact with the virtual world. By implication, VR scenarios also provoke physiological changes in terms of EDA and HR even though the results on HR were not as distinct (Diemer, Mühlberger, Pauli, & Zwanzger, 2014). Interestingly, they cannot only be used to trigger an initial response of the sympathetic nervous system but also to study habituation effects while the participant stays in the virtual environment (Mühlberger, Herrmann, Wiedemann, Ellgring, & Pauli, 2001). When speaking of habituation effects, the parasympathetic nervous system also needs to be mentioned: In simplified terms it can be regarded as the antagonist of the sympathetic nervous system. In this regard, it contributes to the reestablishment of homeostasis after a stress response by also using cholinergic neurotransmission in interplay with muscarine as well as nicotine receptors which causes the initiation of autonomous changes such as bronchoconstriction, vasodilation and the down-regulation of the HR (McCorry, 2007).

A possibility of directly assessing parasympathetic activity is measuring heart rate variability (HRV). HRV includes a number of different parameters which can be analysed in order to gain knowledge about different aspects of an individual´s autonomous nervous system response. In this context, after transforming the HRV from the time to the frequency domain it is especially interesting to differentiate between the low (LF) and the high frequency (HF) components as the LF is generally mediated by the sympathetic as well as the parasympathetic nervous system while the HF is solely mediated by the parasympathetic nervous system. Thus, the ratio of these two sub-measures of HRV can give a good estimation of the interplay of sympathetic and parasympathetic activation (Berntson et al., 1997) and can consequently also be used to describe habituation effects.

1.3.4 Situationally bound and situationally predisposed anxiety

So far, the neuronal network that gets activated at the moment of confrontation with fear-inducing stimuli as well as the associated psychophysiological reactions have been delineated. While there are some situations or stimuli which automatically lead to this response of the "fear network", there are others which just increase the probability of such a reaction. Hence, it is important to differentiate between situationally bound and situationally predisposed anxiety. A classical example of situationally bound fear is a specific phobia where the affected person gets triggered whenever they are confronted with the phobic object and hence tries to avoid the situation in the future or else only handles the confrontation under great emotional distress (American Psychiatric Association, 2013). Hereby, the fear acquisition is thought to operate via classical conditionining (Pavlov, 1927) or observational learning (Olsson, Nearing, & Phelps, 2007; Rosen & Schulkin, 1998) whereby genetic predisposition in terms of a higher susceptibility of some individuals to develop pathological fear (see section 1.3.2) of especially evolutionary relevant stimuli (for example a spider or a snake) certainly also plays a role (Hettema, Neale, & Kendler, 2001). In this context, it is not important whether the person explicitly remembers an aversive event with the phobic object, e.g. being bitten by a dog or watching somebody else being bitten by a dog (Eysenck, 2014). It is, however, essential that the contingency relationship between the stimulus and the fear response is stable and occurs automatically without deliberate influence by the person during the first moment of confrontation (American Psychiatric Association, 2013).

For situationally predisposed anxiety, on the other hand, the contingency relationship between stimulus and response is not a linear one. In this regard, panic disorder can be seen as a typical example of situationally predisposed anxiety. By definition, panic disorder is characterised through reoccurring panic attacks which are accompanied by a number of physiological reactions such as tachycardia, hyperventilation, sweating or nausea. Usually, the first of these panic attacks happens "out of the blue" in an unexpected situation. This way, the person is lacking a logical explanation for the attack and hence may start worrying about possible reasons thereby implicitly attributing a certain danger to the situation where it first occurred (American

Psychiatric Association, 2013). As a consequence, they will probably worry and thus be on higher alert as soon as they get into a similar situation the next time and pay more attention to potential physical signs for another panic attack. This increased vigilance and attention to somatic sensations, on the other hand, does in fact increase the chance to actually experience another panic attack in the situation. Nevertheless, the attack can still not clearly be ascribed to the situation per se, hence more and more similar situations become predisposed for the occurrence of panic attacks (for instance, somebody could have the first panic attack on a bus ride and then transfer this experience to train rides and later to all types of public transportation as the general features of the situation are similar) (Yoris et al., 2015). As situationally predisposed anxiety only means there is an increased chance to actually have a fear response, the reinforcement of the learning experience only takes place intermittently and is therefore also harder to extinguish (Wittchen & Hoyer, 2011) which has relevant implications for its therapy (see section 1.4.2).

Thus, to conclude it needs to be noted that specific phobia and to an even greater extent panic disorder, do not only include the situational fear reaction but also comprise anticipatory anxiety which involves the internal representation of possible (aversive) future events which is mainly associated with prefrontal activation (Holtz, Pané-Farré, Wendt, Lotze, & Hamm, 2012). In fact, regarding the pathogenesis as well as maintaining conditions of anxiety disorders, anticipatory anxiety plays a core role as it usually leads to avoidance of the feared object or situation thereby preventing the affected person from making new learning experiences, e.g. noticing that not all dogs bite or that it is in fact not dangerous to take a train despite having gone through a panic attack there before. Especially in the case of situationally predisposed anxiety it may also happen that instead of complete avoidance a person still frequents the situation but then escapes as soon as they notice any signs (such as increased heart beat) of a fear reaction which may also be described as "fear of fear itself". Analogical to avoidance, escape prevents the person from learning that neither the situation nor the experience of a panic attack in it is life threatening. This interplay of an acute fear reaction, anticipatory anxiety as well as escape and avoidance as maladaptive coping mechanisms finally lead to something which is commonly called "the viscous cycle of anxiety" (Armfield, 2013; Westbrook,

Kennerley, & Kirk, 2011) whereby most contemporary treatment options try to find a way to interrupt it (see next section).

1.4 Contemporary treatment options

1.4.1 Current guidelines

According to the current guidelines for the treatment of anxiety disorders (Bandelow et al., 2014), a number of effective therapy options exist. Regarding panic disorder, psychotherapy as well as pharmacotherapy is recommended whereby, so far, randomised controlled studies showed an effective treatment effect only for CBT rather than psychodynamic therapy. Especially in the case of agoraphobic avoidance behaviour, the therapy should include therapist-guided exposure where the patient confronts his fears with instruction by the therapist. Until now, there is lacking evidence that individual therapy is most beneficial for the patient, so the therapy may also be conducted in a group setting. Referring to pharmacotherapy, especially the administration of selective serotonine re-uptake inhibitors (SSRIs) such as Citalopram, Escitalopram, Paroxetin or Setralin as well as serotonin noradrenalin re-uptake inhibitors (SNRIs) such as Venlafaxin is advised. Even though benzodiazepines effectively reduce acute fear reactions, they should only be administered under special circumstances (for example a severe comorbid cardiovascular condition, otherwise self-endangering behaviour like suicidality) as they are known for their dependence potential. Moreover, they can be seen as a kind of avoidance or escape strategy as they limit or reduce the fear response and impede the acquisition of new information (Vidailhet et al., 1994).

As significant a improvement during the combined treatment of CBT and psychotropic medication in terms of SSRIs and SNRIs as compared to monotherapy could be shown in otherwise treatment-resistant patients with panic disorder (Freire, Zugliani, Garcia, & Nardi, 2016), a combined approach is recommended in such cases. Interestingly, a recent study by Liebscher et al. (2016) compared the effects of CBT with therapist-guided or non-guided exposure with the administration of SSRIs and SNRIs and found that both, psychotherapy as well as pharmacotherapy

lead to a significant reduction of depressiveness and general anxiety symptoms like the number of panic attacks, restlessness or worrying compared to a waiting control group. However, patients that received CBT and particularly therapist-guided exposure training further showed a significantly greater reduction of anticipatory fear and agoraphobic avoidance behaviour which may imply that actually being confronted with a panic-associated situation has a more specific effect on anxiety compared to pharmacotherapy and reduces not only the number of experienced panic attacks per se but also the fear of possibly experiencing one. Thus, this finding may be an explanation for the add-on effect of CBT to sole pharmacological treatment. Apart from these rather "traditional" treatment options, regular endurance sport may also be helpful to fight panic symptoms according to expert opinion and has, therefore, been included into the current guidelines.

Regarding the treatment of specific phobia, the state of the art treatment focuses on exposure therapy whereby the exposure should be conducted in-vivo if possible. Whether it is accomplished in a graduated manner or via "flooding" where the patient is confronted with his or her worst fear from the beginning is not further specified and can therefore be decided depending on the phobic object and the individual patient. If in-vivo exposure is not feasible, the confrontation with the phobic object should be achieved via VR. Even though different studies exist that tried to combine exposure therapy with the administration of pharmacotherapy there is little evidence for a significant add-on effect until now (Abramowitz, Deacon, & Whiteside, 2012).

To conclude, the current guidelines for the treatment of anxiety disorders offer a great number of treatment approaches which have been evaluated with respect to their efficacy. However, up to a third of all patients does not benefit sufficiently (Diemer, Vennewald, Domschke, & Zwanzger, 2010; Freire et al., 2016; Taylor, Abramowitz, & McKay, 2012). For this reason, further research is conducted in order to still improve the standard of knowledge and shall be discussed in the next chapter.

1.4.2 Recent advances in the understanding and treatment of anxiety

1.4.2.1 Investigation of markers predicting therapy response

Regarding the research on the mechanisms of action of therapeutic interventions, an important domain is the knowledge of predictive neurobiological markers which may yield information on the efficacy of a particular treatment and the according clinical decision. Thus, Lueken et al. (2016) performed a systematic review and found that genetic as well as neuroimaging and psychophysiological markers may serve as valid predictive clues on pharmacological but also psychotherapeutical treatment outcome. In more detail, the authors found a possible association between the 5-HTTLPR/rs25531 variant, which influences serotonergic neurotransmission, and treatment response. Intriguingly, the direction of the relationship was determined by the received treatment: while the more active allele was associated with a better response to pharmacotherapy, the less active allele was associated with a better response to psychotherapy which may be explained by the assumption that the latter allele is also linked to increased environmental sensitivity whereby psychotherapy can be considered as a relevant environmental factor.

With respect to markers based on neuroimaging, the most consistent results were found for the ACC as well as for temporal lobe activation. Regarding the ACC, the direction of the relationship between baseline activation and treatment outcome did not only depend on the location within the ACC and the type of treatment but also on the task the patients had to perform, so further research needs to be done to get a better specification. The results for the temporal lobe are also relatively heterogeneous depending on the exact location but it may be assumed that especially visual object processing and recognition might be of predictive value for therapy response.

Psychophysiological measures put the HR as well as HRV and blood pressure in the focus of attention even though, so far, study results are still ambiguous. Comparable to the above mentioned finding, a differentiation between therapy option and the direction of the effect could be detected whereby high HR, high blood pressure and low HRV were associated with a better response to psychotherapy and a low HR, low blood pressure and high HRV were associated with a better response to

pharmacotherapy. As low heart rate variability is thought to be linked to lower environmental adaptability, this could be a reason why psychotherapy, which is supposed to increase cognitive flexibility, is especially effective in this group of patients.

Even though these studies represent first interesting approaches to find predictive markers of therapy response, further research is needed to develop a methodologically sound background in order to eventually use these biomarkers to inform individual clinical decisions. Apart from the research regarding the prediction of therapy response, there is a whole field which deals with the extension or variation of CBT elements as well as the development of alternative (add-on) treatment options. A general overview shall be given during the next sections.

1.4.2.2 The third generation of behavioural therapy

The third generation or "third wave" of behavioural therapy generally describes a movement away from the cognitive focus of the second generation behavioural therapy to a build-up of new experiences within the therapeutic context (Kahl, Winter, & Schweiger, 2012). In this regard, it is generally less focused on straightforward psychological symptom reduction even though this is of course a desired "side effect". The spectrum of evolving treatment options is very heterogeneous and comprises disorder specific techniques as well as treatment options which can be administered independent of the diagnosis. One of the most investigated approaches in this context is probably acceptance and commitment therapy (ACT) which has originally been developed by Steven Hayes who suffered from "treatment-resistant" panic disorder himself. In this section, it shall therefore be described in more detail as an example of a "third wave" approach in the treatment of anxiety disorders as it further comprises a number of "typical" third wave methods like acceptance-based strategies as well as possibilities on how to pursue a value oriented life depite persisting difficulties.

Being a psychologist himself, when Hayes noticed he did not profit sufficiently from his treatment of panic symptoms, he tried to find alternatives for dealing with them

which included meditation and mindfulness. This way, he learnt to detach his thoughts from his self and hence witness his thinking from the perspective of an observer and to learn how to accept that he was not able to control his panic symptoms permanently (Cloud, 2006). During the following years, Hayes investigated his personal observation of what was helpful in a more scientific framework and developed relational frame theory which may be seen as the basis for ACT. Relational frame theory thereby principally assumes that human language and cognition are relational entities implying that the relationship between stimuli is not just based on the physical properties of the stimuli but also on contextual information. The number of built relations is thereby arbitrary and depends on the social context. Eventually, this signifies that the use of language influences the predications humans make about their environment thereby manipulating thoughts, emotion and finally behaviour (Cullinan & Vitale, 2009). Getting back from relational frame theory to psychotherapy it can be assumed that automated, inflexible relations may lead to psychopathology (Tonneau, 2004). Hence, the general goal of ACT is to increase psychological flexibility by increasing awareness of personal relations in terms of thoughts or experiences without attachment to them. To do so, it uses strategies like mindfulness exercises, cognitive defusion techniques and metaphors. Furthermore, it includes commitment and behaviour change processes whereby the goal is not so much symptom reduction itself but rather accepting the negative experiences one might have while at the same time building a value-based life (Hayes, 2006). Regarding the treatment of anxiety disorders, ACT seems to be equivalently effective as standard CBT. However, the mechanisms of action appear to differ whereby compared to standard CBT, therapy outcome was rather associated with "acting with awareness", "acceptance" as well as the reduction of "experimental avoidance" in ACT treated patients only (Forman, Herbert, Moitra, Yeomans, & Geller, 2007). Thus, it may be generally concluded that ACT is not superior to standard CBT but that there are specific elements which may help patients that would otherwise not benefit. Of course, depending on the patient's individual needs, these elements can be included separately into any psychotherapy. In fact, one might argue that a lot of these interventions have already been used before without being given a specific name and should therefore not be considered "new wave". To

take it further, it may even be reasoned that the "third wave" is actually a step back to the "first wave" as it is generally based on building new relations to the experienced environment by behavioural and emotional activation within the therapeutical context. However, no matter whether the third wave should really be called a new generation or rather "old wine in a new bottle", it includes a diversity of different intervention techniques which – when used deliberately – open up different possibilities for patients which might otherwise not benefit enough. However, further research is needed to achieve a better empirical support (Kahl et al., 2012).

1.4.2.3 New approaches to exposure therapy

As stated above, therapy-guided exposure is one of the first-line treatment options for anxiety disorders, yet clinically significant improvement is only described in 50-65% of all patients (Gloster et al., 2011). Furthermore, it is a common finding that exposure-based methods are applied relatively rarely by therapists despite the recommendation in the guidelines (Levita, Duhne, Girling, & Waller, 2016). Thus, there is still a need of research in order to improve its efficacy, but also reduce the barriers that keep therapists from using it. In this regard, a recent study by Harned, Dimeff, Woodcock & Contreras (2013) tried to predict the adoption of exposure therapy and found that its proficient use was related to the anxiety sensitivity, attitude and expertise of the therapists as well as organisational (e.g., work context, availability of supervision) and patient related factors (like comorbid disorders, resistance or the severity of the symptoms). Interestingly, however, the study could show that most of these factors were moderated by the training the therapists had received whereby a more intensive training could compensate for the originally limiting factors. Even though this result seems rather intuitive, it also implies that the practical training therapists obtain needs to be improved and research in exposure therapy also includes the investigation of the factors that make the training most effective so that it will be actively adopted. Naturally, research in the field of exposure therapy does not only comprise treatment delivery factors but also its mode of action per se. For example, there are studies (for example, Craske et al.,

2008) that suppose that not fear reduction during the exposure situation but rather fear tolerance, meaning the absence of experimental avoidance (e.g., dysfunctional emotion regulation strategies like suppression), predict long-term success of exposure therapy. In this regard, patients might actually benefit more if they can leave the feared situation and end the exposure therapy before anxiety has decreased (provided they resume exposure at a later time) rather than enduring the aversive event until the end. On a similar account, the use of safety signals still needs to be investigated further as, on the one hand, one may deduce that as long as safety signals are present the missing occurrence of a feared event (such as fainting or losing control) can always be attributed to the presence of the safety signal, hence new learning that the situation per se is not dangerous does not seem possible. On the other hand, however, one may argue that it is rather the (cognitive) occupation with the safety signal that inhibits new learning when it is used as some kind of avoidance strategy and as long as that is prevented safety signals may also help the patient to tolerate the fear at the beginning of exposure therapy. Even though, as mentioned in section 1.4.1, studies on the combination of exposure therapy and pharmacotherapy to improve extinction learning (extinction not meaning "unlearning" but rather the active aquistion of new knowledge which finally prevents or at least temporarily diminishes the response to a conditioned stimulus (Phelps, Delgado, Nearing, & LeDoux, 2004)) do exist, so far there does not seem to be a significant add-on effect. However, apart from classical pharmaceutical administration, recent research deals with the combination of exposure and different new agents also referred to as "neuroenhancers". The underlying presumption of combining neuroenhancers and exposure therapy is that these agents facilitate the forming of new memories by, for example, targeting NMDA receptors and therefore boostering habituation processes and extinction learning. In a recent review (Hofmann, Mundy, & Curtiss, 2015), the authors compared the effect of a number of commonly studied substances such as d-cycloserine, yohimbine, cortisol, catecholamines, oxytocin, modafinil as well as some nutrients like caffeine and amino fatty acids and found that d-cycloserine showed the most promising result. Nevertheless, they argue that further studies are needed to investigate the optimal timing and dosage as well as the long-term effects of its usage. Moreover, as the

endocannabinoid system is thought to play an important role in the modulation of PFC activity (see section 1.3.2), there are also approaches to administer substances which make the non-psychotominetic component cannabidiol of cannabis available within the brain which, besides its anxiolytic effects, also presumably facilitates extinction learning (Das et al., 2013; Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015).

Another factor, which deserves some consideration when planning exposure therapy, is the level of control over the environment as well as the related costs. For instance, even though it has been known for a long time that exposure therapy is a very effective treatment option for flight phobia (Haug et al., 1987), it is rather expensive to conduct repeated exposure sessions on a plane. Moreover, the conditions (for example bad weather) during the flight cannot be manipulated according to the patients' needs by the therapist. In this regard, a lot of current studies focus on the effectiveness of VR exposure therapy (Powers & Emmelkamp, 2008). Besides the visual input the patient receives over the HMD or a special computer monitor, further sensory input such as acoustic, haptic or olfactory stimulation may also be included to simulate the presence in the virtual environment and to allow interaction with it (Mühlberger et al., 2001). Meanwhile, the effectiveness of VR exposure has been established by independent meta-analyses (Morina, Ijntema, Meyerbröker, & Emmelkamp, 2015; Opriş et al., 2012), but still larger controlled studies as well as further research regarding factors like the specifics in terms of the treated type of phobia, the combination of VR and real world elements (Baus & Bouchard, 2014) or the influence of the sense of presence during VR immersion (Morina et al., 2015) are needed to further establish VR as an efficacious treatment tool. Interestingly, regarding the latter point, there seems to be a positive correlation between the sense of presence and perceived anxiety, at least when using VR in the treatment of animal phobia, while for social phobia such a relation has not been shown until now (Morina et al., 2015).

1.4.2.4 Neurostimulation and neurofeedback – a neurophysiologically based treatment perspective

Both neurostimulation as well as neurofeedback take the existing findings on anxiety disorders regarding alterations within the fear network as the basis for their approach. The underlying idea of neurofeedback is that patients learn to consciously control their brain activation patterns by basically receiving a real-time visual feedback of their ongoing brain activation over a display and having to find a way to self-regulate it in a particular way according to a given instruction (Gevensleben et al., 2014).

For instance, regarding the found prefrontal hypoactivation which is related to pathological fear reactions, it might be an option to instruct the patient to regulate specific parameters which are associated with the brain's activational state whenever a certain (possibly fear-inducing) stimulus is presented. To do so, a number of different feedback methodologies including electroencephalography (EEG), functional magnetic resonance imaging (fMRI) or functional near-infrared spectroscopy (fNIRS) are possible (Mayer, Wyckoff, Fallgatter, Ehlis, & Strehl, 2015). Especially regarding the treatment of attention deficit hyperactivity disorder (ADHD) multiple studies already exist which could show an improvement of clinical symptoms (Gevensleben et al., 2014) but also significant alterations with regard to the underlying neural activation patterns (Lévesque, Beauregard, & Mensour, 2006) which suggests that the neurodfeedback approach might generally also be suited for other psychiatric disorders. In this context, a recent controlled study (Zilverstand, Sorger, Sarkheil, & Goebel, 2015) which investigated fMRI-based neurofeedback training over the left DLPFC and right insula in a group of spider phobics could show a reduction of subjectively perceived anxiety as well as changes in insula activation in the neurofeedback training group. Hence, from this perspective it seems promising to further investigate neurofeedback training as a therapeutic tool in anxiety disorders.

Another way to purposefully influence brain activation in distinct areas is neurostimulation. In this context, two common methods comprise rTMS as well as transcranial direct current stimulation (tDSC). Both methods can be considered non-invasive in the sense that the neurostimulation is achieved from outside through the skull without the necessity of implanting micro-electrodes into the brain. To do so,

rTMS uses a specific coil placed on the skull above the chosen area which produces electric pulses in order to cause the depolarisation of the underlying neurons and hence the discharging of action potentials via electromagnetic induction. Depending on the frequency of the electric and resulting magnetic pulses a facilitory or inhibiting effect may be achieved (Vennewald, Diemer, & Zwanzger, 2013).

Theta burst stimulation (TBS) is a refined form of rTMS which again may be applied in an activating (intermittent, iTBS) or inhibitory (continuous, cTBS) fashion. In comparison to traditional rTMS, its advantages include that a longer lasting stimulation effect may be achieved after a shorter stimulation time (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). Accordingly, studies could demonstrate acute TBS effects on cortical activation of the underlying areas which lasted about one hour after one-time application (Grossheinrich et al., 2009). Having said this, it must however be kept in mind that TBS, or more generally rTMS application over one specified area, may also affect other brain regions (Ilmoniemi et al., 1997). Indeed, some studies exist which showed that rTMS can cause the opposite effect on the contralateral hemisphere (Di Lazzaro et al., 2008). TDCS, on the other hand, uses two electrodes which are attached on the scalp in order to create a constant low current in-between which increases (anodal stimulation) or decreases (cathodal stimulation) the threshold for action potentials in the underlying neuronal organisations (Nitsche & Paulus, 2001). While the advantage of rTMS is a better spatial resolution (Antal, Nitsche, & Paulus, 2006), tDCS is more user-friendly as the stimulation is hardly noticeable and can hence be accomplished while the patient is involved in other activities (e.g. simultaneous application during therapy session (Bajbouj & Padberg, 2014). Recent studies could even show a beneficial effect of the combination of the two methods which surely also presents a further field of future research. Generally, especially in repeated applications, rTMS as well as tDCS are supposed to initiate long-lasting changes in cortical excitability which are thought to be achieved via the associated neurotransmitter release and thus neuroplastic processes (Gersner, Kravetz, Feil, Pell, & Zangen, 2011). Regarding their clinical application in the treatment of anxiety disorders, so far only a few randomised controlled studies exist for either of these methods. However, a systematic review (Kekic, Boysen, Campbell, & Schmidt, 2016) which evaluated the use of tDCS in

psychiatric disorders found an exponential increase over the last ten years whereby the study outcome suggested a beneficial effect of repeated tDCS application on symptom severity. However, the main research focus seemed to be on depressive disorders (Shiozawa, Fregni, et al., 2014). For anxiety disorders, only very few studies or case reports exist (Kar & Sarkar, 2016; Shiozawa, Leiva, et al., 2014) which nevertheless showed some encouraging effects. Further, one study (van't Wout et al., 2016) could demonstrate a favourable add-on effect of tDCS during exposure-based extinction learning. Even though more studies examining the impact of rTMS on anxiety symptoms exist, again there is a lack of randomised controlled studies and results are inconsistent as has been demonstrated in a systematic review by Zwanzger, Fallgatter, Zavorotny & Padberg (2009) or even more recently by Vennewald et al. (2013). In this respect, in line with the above quoted valence hypothesis most studies or rather case reports and clinical trials used either facilitory stimulation on the left hemisphere (Dresler et al., 2009; Guaiana, Mortimer, & Robertson, 2005) or inhibitory stimulation on the right hemisphere (Mantovani, Aly, Dagan, Allart, & Lisanby, 2013; Schutter, van Honk, d'Alfonso, Postma, & de Haan, 2001) in panic disorder and found an improvement of clinical symptoms. However, sample sizes are often small and the results are inconclusive for example Prasko et al. (2007) and Vennewald et al. (2016) found no difference on fear processing between sham and active inhibitory rTMS over the right PFC). Further, there is a lack of studies which examined a possible add-on effect of rTMS to (exposure-based) psychotherapy. In fact, when considering that it does not only influence the brain's current activational state but also fosters neuroplastic processes by means of neurotransmitter release (Gersner et al., 2011), it may, comparable to neuorenhancers, serve as a tool to increase the effects of new learning experiences during psychotherapy. The assumption that it might be promising to further pursue this idea of employing rTMS not as a stand-alone therapeutic tool but rather an add-on to (exposure-based) psychotherapy is supported by a preliminary report by (Osuch et al., 2009) who showed that active compared to sham rTMS improved the effects of imaginal exposure therapy and reduced physiological hyperarousal in a sample of patients suffering from chronic post-traumatic stress disorder (PTSD).

1.5 Rationale for the current work

The previous sections have given an update on the theoretical background regarding pathological fear and associated neurobiological findings on the one hand, and its current treatment recommendations and their development on the other hand. Accordingly, the present work intended to combine the knowledge of both domains in order to investigate the possibility to directly influence the underlying neural activity related to anxiety disorders in order to alleviate clinical symptoms. To do so, two different studies were conducted where we examined the effects of a neuromodulation technology on emotional as well as cognitive aspects of anxiety in more detail.

Regarding the first study, in line with the valence hypothesis, we chose to apply a sham-controlled activating rTMS protocol over the left PFC in a group of patients suffering from panic disorder with or without agoraphobia during a time course of three weeks as an add-on to manual-based CBT which took place in a group setting. Before the start as well as after the completion of the rTMS administration, a measurement of functional brain activation by means of fNIRS was conducted while the patients completed an emotional (Emotional Stroop task) as well as a cognitive (Verbal fluency task) paradigm. In order to validate the valence hypothesis as well as the effect of rTMS on prefrontal functioning, we also investigated a group of healthy controls with the same two paradigms. As the studies on neurostimulation described in the sections above found some promising results with respect to its effects on anxiety, we attempted to not only quantify its impact per se but rather aimed at investigating whether it might accelerate or reinforce the effects of CBT. In more detail, we addressed the following research hypotheses:

(1) In line with the currents findings on alterations within the fear network, patients suffering from panic disorder with or without agoraphobia significantely differ relative to healthy controls regarding their prefrontal activation during the completion of an emotional paradigm.

(2) These differences in activation patterns are not only present during emotional tasks but, more generally, may rather be a characteristic in this group of patients

and therefore also be detectable during cognitive tasks in terms of prefrontal hypoactivation as predicted by the valence hypothesis.

(3) RTMS has the ability to specifically enhance these prefrontal activation patterns.

(4) An increase in activation should be correlated with clinical symptom reduction and thus treatment efficacy of CBT.

During the second study, we decided to again apply a sham-controlled activating rTMS protocol at the same stimulation site. However, this time we aimed at eliminating as many confounding effects as possible (e.g. simultaneous psychotherapy) and therefore investigated a group of spider phobic subjects that did not suffer from any comorbid psychological disorders whereby they received their treatment just prior to a phobia-related VR challenge which served as the fear-inducing situation. Before as well as right after rTMS application in combination with the VR challenge their prefrontal brain activation was again recorded by means of fNIRS during an emotional Stroop paradigm. As in the first study, the effects within the phobic group were compared to the effects of a healthy control group. The postulated research hypotheses were framed as the following questions:

(1) Being confronted with virtual spiders will provoke anxiety as well as disgust which is associated with the activation of the sympathetic nervous system (increase in HR and EDA as well as alterations in HRV) in people suffering from spider phobia.

(2) These reactions are less prominent in healthy control subjects.

(3) Prefrontal activation patterns of spider phobics should differ from the ones of healthy control subjects when responding to phobia-related stimuli.

(4) Accordingly, in comparison to the healthy control group, the performance of the spider phobics should be reduced (increased reaction times, higher error rates).

(5) Active rTMS over the prefrontal cortex should lead to improved cognitive control and therefore attenuate the increase of anxiety and disgust. This effect should be detectable for all associated measures (activation of the sympathetic nervous system, brain activation, behavioural performance, perceived valence and arousal of the presented stimuli).

(6) Nevertheless, participants with spider phobia should still experience a more pronounced feeling of presence during the VR challenge due to their residual fear.

(7) Finally, to get a better understanding of the emotional control processes on a neuronal level, we conducted a functional connectivity analysis within the prefrontal cortex in addition to the standard fNIRS analysis.

2. Study 1: Clinical and neurobiological effects of NIRS-controlled transcranial magnetic stimulation (rTMS) in patients with panic disorder during CBT treatment

2.1 Manuscript 1: Does rTMS alter neurocognitive functioning in patients with panic disorder/agoraphobia? – An fNIRS-based investigation of prefrontal activation during a cognitive task and its modulation via sham-controlled rTMS

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2.1.1 Abstract

Objectives. Neurobiologically, panic disorder (PD) is supposed to be characterised by cerebral hypofrontality. Via functional near-infrared spectroscopy (fNIRS), we investigated whether prefrontal hypoactivity during cognitive tasks in PD-patients compared to healthy controls (HC) could be replicated. As intermittent theta burst stimulation (iTBS) modulates cortical activity, we furthermore investigated its ability to normalise prefrontal activation. *Methods.* Forty-four PD-patients, randomised to sham or verum group, received 15 iTBS-sessions above the left dorsolateral prefrontal cortex (DLPFC) in addition to psychoeducation. Before first and after last iTBS-treatment, cortical activity during a verbal fluency task was assessed via fNIRS and compared to the results of 23 HC. *Results.* At baseline, PD-patients showed hypofrontality including the DLPFC, which differed significantly from activation patterns of HC. However, verum iTBS did not augment prefrontal fNIRS activation. Solely after sham iTBS, a significant increase of measured fNIRS activation in the left inferior frontal gyrus (IFG) during the phonological task was found. *Conclusion.* Our results support findings that PD is characterised by prefrontal hypoactivation during cognitive performance. However, verum iTBS as an "add-on" to psychoeducation did not augment prefrontal activity. Instead we only found increased fNIRS activation in the left IFG after sham iTBS application. Possible reasons including task-related psychophysiological arousal are discussed.

2.1.2 Introduction

According to DSM-IV, panic disorder (PD) is characterised by the sudden onset of unexpected panic attacks resulting in constant worries about possible reasons and negative consequences of the attacks. Moreover, in the case of comorbid agoraphobia, this eventually leads to behavioural avoidance of situations from which escape might be difficult in case of an attack (American Psychiatric Association, 2000). On a neurobiological level, functional imaging studies of PD-patients with and without agoraphobia have found hypoactivity of the prefrontal cortex (PFC), paired with hyperactivity of fear relevant brain structures such as the amygdala, suggesting an inadequate inhibition by the PFC in response to anxiety-related stimuli (Dresler et al., 2013; Gorman et al., 2000; Gorman, Liebowitz, Fyer, & Stein, 1989). In fact, hypofrontality of PD-patients has not just been observed in response to emotional stimuli (Dresler et al., 2012), but also during cognitive tasks without any emotional content. For example, in a near-infrared spectroscopy study, Nishimura et al. (Nishimura et al., 2007) reported hypoactivation of the left PFC in particular while Otha et al. (2008) found that PD-patients as well as patients with a depressive disorder showed lower bilateral prefrontal activation than healthy controls during a verbal fluency task. Moreover, Nishimura et al. (2009) investigated a potential relation between the frequency of panic attacks/agoraphobic avoidance and PFC activation during a cognitive task, indeed finding an association between altered activation patterns in the left inferior prefrontal cortex and panic attacks as well as between the anterior part of the right PFC and the severity of agoraphobic avoidance.

Cortical activation patterns can be selectively modified by means of repetitive transcranial magnetic stimulation (rTMS) via electromagnetic induction (Wassermann & Zimmermann, 2012). This way, rTMS has been shown to modulate neurotransmitter release (Pogarell et al., 2007) and—depending on its stimulation frequency—normalise prefrontal hypoactivity (Speer et al., 2000). In fact, even though results are still inconsistent (Herwig et al., 2007), rTMS has been shown to have a moderate antidepressant effect (Rumi et al., 2005; Schutter, 2009). Within this framework it is of special interest that the method does not just seem to alter

affective states but also cognitive functioning (Cho, Yoon, Lee, & Kim, 2012; Yamanaka, Yamagata, Tomioka, Kawasaki, & Mimura, 2010).

Functional near-infrared spectroscopy (fNIRS) is an imaging method which allows for a less complicated and faster application compared to other imaging methods such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) (Ernst, Schneider, Ehlis, & Falgatter, 2012). Especially psychiatric patients with claustrophobic fears benefit from the fact that they merely need to sit in a chair while optodes that emit and receive near-infrared light are attached to their heads (Irani, Platek, Bunce, Ruocco, & Chute, 2007). This way, task-related changes in oxygenated and deoxygenated haemoglobin concentrations can be examined. Even though disadvantages such as a relatively low spatial resolution (approximately 3 cm), a limited penetration depth (approximately 2 to 3 cm) (Cui, Bray, Bryant, Glover, & Reiss, 2011; Haeussinger et al., 2011), and influences of extracranial signals do exist (for a review see (Ferrari & Quaresima, 2012), fNIRS has proven to be a useful tool in psychiatric research (Ehlis, Schneider, Dresler, & Fallgatter, 2014).

Based on these findings and considerations, the goal of the current study was to (1) clarify whether the findings of Otha et al. (2008) concerning prefrontal hypoactivity in PD-patients compared to healthy controls during a cognitive paradigm (verbal fluency task) could be replicated via fNIRS in a larger sample. Also, a sham-controlled rTMS protocol was applied over the time course of three weeks above the left DLPFC to (2) examine whether excitatory rTMS can serve as an adequate tool in order to improve cognitive dysfunction in terms of prefrontal hypoactivation in PD-patients. In this regard, the patients' behavioural performance during the verbal fluency task was also taken into account.

2.1.3 Materials and methods

2.1.3.1 Participants

Patients were recruited via the outpatient departments of the two study centres, advertisement in newspapers, as well as the internet and information material sent

to local physicians. Exclusion criteria for all participants were age under 18 and over 65 years, pregnancy, and severe somatic disorders (e.g., cardiovascular disease, epilepsy, and neurological disorders). Also, patients fulfilling rTMS contraindications such as ferromagnetic implants or significant abnormalities in routine EEG were excluded. All patients were diagnosed with PD with or without agoraphobia according to DSM-IV-TR criteria (American Psychiatric Association, 2000). Nonprominent comorbid psychiatric disorders (except for bipolar or psychotic disorder, borderline personality disorder, acute substance abuse disorders, and acute suicidality) were no exclusion criteria. Psychopharmacological treatment was permitted if the dosage had been stable for at least three weeks prior to baseline assessment (*t*1). Benzodiazepines, tricyclic antidepressants (except for Opipramol), and antipsychotics (expect for Quetiapine with maximal dosage of 50 mg) were excluded. Healthy controls who suffered from any axis-I psychiatric disorder (except for specific phobia) or had a family history of psychiatric disorders were excluded. A total of 23 controls and 44 PD-patients, of which 22 were randomised to the sham and 22 to the verum rTMS group, were selected for the study. Groups did not differ with respect to gender, age, years of education, and handedness (Table 1). After a comprehensive study description, written informed consent was obtained. The study was approved by the Ethics Committees of the Universities of Muenster and Tuebingen and all procedures were in accordance with the latest version of the Declaration of Helsinki.

Table 1: Sociodemographic sample characteristics.

Group	Age mean (range)	Gender	Handed-ness	First language	Years of education Mean (SD)	Duration of Illness in months Mean (range)
Controls	33.4 (19-64)	14 females 9 males	20 right 3 left	22 German 1 bilingual	12.5 (1.1)	-
Sham	36.3 (22-56)	14 females 8 males	21 right 1 left	20 German 1 bilingual 1 other	12.4 (2.0)	84 (1-336)
Verum	37.6 (19-63)	13 females 9 males	20 right 2 left	19 German 1 bilingual 2 other	12.1 (1.7)	92 (1-372)

Comparisons						
Controls vs. Sham	t_{43}=-0.921 p=0.362	χ^2_1=0.037 p=0.848	χ^2_1=1.003 , p=0.317	χ^2_2=1.531 p=0.465	t_{33}=-0.234 p=0.816	-
Controls vs. Verum	t_{43}=-1.148 p=0.257	χ^2_1=0.015 p=0.903	χ^2_1=0.178 p=0.673	χ^2_2=2.198 p=0.333	t_{37}=-0.913 p=0.367	-
Sham vs. Verum	t_{42}=-0.399 p=0.692	χ^2_1=0.096 p=0.757	χ^2_1=0.358 p=0.550	χ^2_2=0.667 p=0.717	t_{42}=0.490 p=0.626	t_{42}=-0.290 p=0.773

SD: standard deviation.

2.1.3.2 Design

PD-patients received a total of 15 rTMS applications during three weeks at one of the study centres (Muenster or Tuebingen). Before the first and after the last rTMS-session brain activation was assessed with fNIRS while patients were performing a cognitive task. Between the first and the second fNIRS assessment, all patients received three group sessions of psychoeducation concerning PD. Healthy control subjects attended the two fNIRS measurements but received no rTMS in-between. Enrolment took place between January 2011 and July 2013. Patients and therapists were blinded to rTMS group assignment. This investigation was conducted within the framework of a larger study which included 9 weeks of cognitive behavioral therapy for patients with panic disorder/agoraphobia and additional fNIRS investigations described elsewhere (Deppermann et al., submitted, see section 2.2).

2.1.3.3 Psychoeducation

Psychoeducation sessions were held in groups of up to 6 participants and were conducted by trained psychologists, who were supervised regularly by clinical psychotherapists. A state-of-the-art, standardised treatment manual was used (Margraf & Schneider, 1998, 2013). The content of the sessions included information about the pathogenesis of PD and agoraphobia, the vicious cycle of anxiety, somatic components of anxiety, and the sharing of personal experiences among the patients.

2.1.3.4 Verbal fluency task (VFT)

All subjects were assessed twice within a three-week interval between the first (*t*1) and the second (*t*2) measuring time.

During the measurements participants sat in a comfortable chair and were advised to keep their eyes closed and relax in order to avoid head or body movements. The VFT consisted of a phonological, a semantical and a control task. During the phonological task, subjects were instructed to produce as many nouns as possible beginning with a certain letter, whereas during the semantical task they had to name as many nouns as possible belonging to a certain category while repetitions and proper nouns were supposed to be avoided. During the control task the participants were instructed to repeat the weekdays in a speed that approximately matched the number of recited days to the number of mentioned nouns. The VFT started with a resting state phase of 10 seconds followed by the different tasks and more resting state periods, which lasted 30 seconds each. The sequence of the three tasks and resting phases were repeated three times, each time with a different letter or category. The letters and categories were chosen from the "Regensburger Wortflüssigkeitstest" (Aschenbrenner, Tucha, & Lange, 2000). Different letters/categories were used at *t*1 and *t*2 and counterbalanced between subjects. During the resting phase, participants were told to relax.

2.1.3.5 rTMS

Starting after the first fNIRS measurement, intermittent theta burst stimulation (iTBS, Huang et al., 2005) was applied in the patient group during 15 daily sessions on workdays during three weeks with a figure-of-eight coil (MCF-B65, 2 × 75 mm diameter, n = 34, MAGSTIM 9925-00, 2 × 70 mm, n = 9) by means of a MagOption/MagPro X100 stimulator (MagVenture, Denmark, n = 34) and a MAGSTIM RAPID2 T/N 3567-23-02 stimulator (n = 9), respectively. ITBS was used in order to achieve a facilitating effect on cortex excitability, as this could be demonstrated for the motor cortex, but also for more frontal cortex areas in previous studies (Huang et al., 2005; Restle, Murakami, & Ziemann, 2012). The iTBS protocol consisted of a

total of 600 pulses applied in intermittent biphasic bursts at a frequency of 15 pulses per second via 2 second trains, starting every 10 seconds as described by Huang et al. (2005). The time of day for iTBS application did not vary for more than 2 hours from one day to the next. As the circadian rhythm is known to influence cortical excitability (Sale, Ridding, & Nordstrom, 2007) the participants' individual resting motor threshold was determined prior to each iTBS session on the left motor cortex and stimulation intensity was set to 80% of this threshold. Stimulation site was F3 (left DLPFC) according to the international 10–20 system for electrode placement (Herwig, Satrapi, & Schönfeldt-Lecuona, 2003). In order to ensure that the site of stimulation stayed constant over all sessions, F3 was drawn onto an individual textile cap for each participant prior to the first session. Additionally, other orientation points as the nasion, the inion, and the auricles were sketched on. While the coil was held tangentially to the scalp forming a 45° angle to the mid-sagittal line of the head (handling pointing in a posterior direction) for verum stimulation, it was flipped away from the scalp in a 90° angle for the sham stimulation. The post-fNIRS measurement (t2) was set to be conducted no earlier than 12 hours after the last rTMS-session to avoid the measurement of acute rTMS effects.

2.1.3.6 fNIRS

Relative temporal changes in oxygenated (O2Hb) and deoxygenated haemoglobin (HHb) were measured from a 10-second baseline using the ETG-4000 optical topography system (Hitachi Medical Co., Japan). For this purpose, the ETG-4000 uses laser diodes which emit light of two wavelengths (695 ± 20 nm and 830 ± 20 nm) and photodetectors which receive the scattered light intensity. Since the main light absorbers in this setup are the two types of haemoglobin, changes in measured light intensity between the emitter-detector pairs can be related to haemodynamic changes—which are coupled to neural activation—using a modified Beer-Lambert equation (Obrig & Villringer, 2003). Altogether the probe set consisted of 16 photodetectors and 17 light emitters arranged in a 3 × 11 fashion with an interoptode distance of 3 cm resulting in 52 distinctive channels with a penetration

depth of approximately 2 to 3 cm (Cui et al., 2011; Haeussinger et al., 2011). The probe set was attached over the participants' prefrontal cortex having the central optode of the lowest row on FPz stretching out towards T3 and T4, respectively, according to the 10–20 international EEG system (Jasper, 1958b). The sampling frequency was 10 Hz. The unit used to quantify haemoglobin concentration changes was mmol × mm. Subsequently, the recorded data were averaged over the corresponding blocks and exported into Matlab R2012b (The Math Works Inc., Natick, USA) where they were first corrected for changes in the NIRS signal that were not directly due to functional changes in haemoglobin concentration related to the attended tasks. To this end, frequencies that exceeded 0.05 Hz were removed using a low pass filter and clear technical artefacts (e.g., due to an optode losing contact to the scalp during measurement) were corrected by means of interpolation by replacing the values of the corresponding channels with the values of the circumjacent channels in a Gaussian manner (closer channels were taken more into account). In order to further remove artefacts, due to head movements, a correlation-based signal improvement (CBSI) procedure according to Cui, Bray & Reiss (2010) was applied, adjusting the values for each channel by the equation

$$[\mathrm{CBSI}] = 0.5*([\mathrm{O2Hb}]-\mathrm{std}[\mathrm{O2Hb}]\mathrm{std}\ [\mathrm{HHb}]*[\mathrm{HHb}]).$$

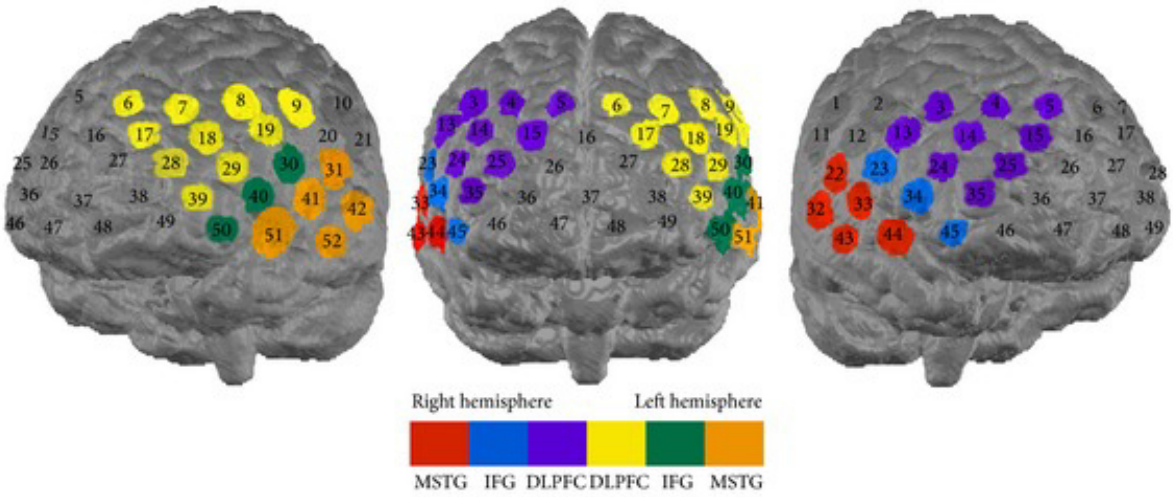
According to this approach, cortical activation should result in a negative correlation between O2Hb and HHb concentrations so in case of positive correlations the O2Hb signal is adjusted. Even though exceptions regarding a strictly negative correlation during brain activation exist (Yamamoto & Kato, 2002), Brigadoi et al. (2014) showed promising results for this procedure. Finally, the CBSI adjusted signal was once more interpolated in a Gaussian manner by using an inner-subject variance threshold of 4 as an interpolation criterion, assuming that exceeding values were most likely the result of further artefacts. Altogether a total of 5% of all channels were replaced.

After preprocessing, the data were averaged for all three groups within a time frame of 0–45 seconds after the onset of each task. The amplitude integrals in CBSI concentration between 5 and 40 seconds were taken as the basis for statistical analysis as a delay of the haemodynamic response after task onset can be assumed.

2.1.3.7 Regions of interest (ROI)

Based on prior studies investigating verbal fluency (Nishimura et al., 2007; Nishimura et al., 2009; Ohta et al., 2008; Schecklmann et al., 2008; Tupak et al., 2012), different a priori ROIs were defined. Accordingly, in addition to temporal areas (middle and superior temporal gyrus (MSTG)) and the inferior frontal gyrus (IFG) comprising Broca's area, the DLPFC is also supposed to be critically involved when performing a VFT. Corresponding channels were chosen using a virtual registration procedure as described by Lancaster et al. (2000), Rorden & Brett (2000) and Tsuzuki et al. (2007) (cf. Figure 1).

Figure 1



Probe set arrangement with numbers indicating channels. DLPFC: dorsolateral prefrontal cortex, IFG: inferior frontal gyrus, MSTG: middle superior temporal gyrus, color-coded channels were used for analyses.

2.1.3.8 Clinical assessment

PD with or without agoraphobia was diagnosed by experienced clinical psychologists with the German version of the Structured Clinical Interview for DSM-IV, Axis I Disorders (SCID-I First, Spitzer, Gibbon, & Williams, 1995; Wittchen, Wunderlich, Gruschwitz, & Zaudig, 1997). Anxiety was measured with the following

questionnaires: Panic and Agoraphobia Scale (PAS; Bandelow, 1999), Hamilton Anxiety Rating Scale (HAM-A; Hamilton, 1996), and Cardiac Anxiety Questionnaire (CAQ; Eifert et al., 2000; Hoyer, Helbig, & Margraf, 2005). All questionnaires were completed at t1 and t2. For all scales, higher scores indicate more severe symptoms.

In case of missing questionnaire items, a last observation carried forward analysis (LOCF) was conducted. If less than 10% of all items were left out, missing values were substituted by the participant's mean on the relevant scale.

2.1.3.9 Statistical analyses

All analyses were conducted with IBM SPSS Statistics 20 and 21, respectively. The sample characteristics were assessed by means of $\chi 2$ tests (gender, handedness, and first language) or t-tests (age, years of education, duration of illness for patients, and questionnaire data for t1 and t2), directly comparing the experimental groups (active versus sham, sham versus controls, and active versus controls). If numbers for the corresponding categories were below 5, Fisher's exact test was considered instead of asymptotic significance. The effects of patients' blinding regarding rTMS treatment condition were evaluated using binomial tests (test proportion: 0.5) for the subjectively perceived rTMS condition in each patient group, separately. The optimal sample size was determined based on previous studies investigating the effect of high-frequency rTMS on symptom severity in depression (e.g., Berman et al., 2000). The effect size of such a treatment protocol was estimated to approximate 0.5, while power was defined as 80%. The α-level was set to 5%. Since the effect of rTMS protocols in patients suffering from anxiety disorders is still difficult to quantify (Pallanti & Bernardi, 2009), it was decided to follow a more conservative assessment resulting in a target sample size of $n = 40$ patients.

For baseline assessment, fNIRS-data for all ROIs were analysed by means of analyses of variance (ANOVA) with the between-subject factor group (patients versus controls). The corresponding behavioural performance was analysed accordingly. In order to verify that changes in CBSI concentration were task-related, effects of hemispheric lateralisation were further analysed using a 2×3 repeated

measurement ANOVA (RM-ANOVA) with the within-subject factors hemisphere (left versus right) and task (semantical versus phonological versus control task). As the factor time was of no relevance within this context, the corresponding data were averaged across the two measurement times. Accordingly, the phonological and semantical task should elicit a left lateralisation in the language relevant ROIs (IFG & MSTG) (Tupak et al., 2012).

To evaluate the effects of rTMS on prefrontal activity, 2 × 3 RM-ANOVAs for each ROI and cognitive task were conducted (within-subject factor time (t1 versus t2), between-subject factor group (verum versus sham versus controls)).

The total number of produced nouns for the phonological and semantical task was investigated according to the collected fNIRS-data via a 2 × 3 RM-ANOVA with the within-subject factors time (t1 versus t2) and the between-subject factor group (verum versus sham versus controls). The number of weekdays was not considered in the analysis as it was matched to fit the number of nouns in the other tasks.

In case of violations of the sphericity assumption, the degrees of freedom in the ANOVAs were corrected using the Greenhouse-Geisser or Huynh-Feldt procedure depending on ε (ε > 0.75 Huynh-Feldt, ε < 0.75 Greenhouse-Geisser; (see Quintana & Maxwell, 1994). To avoid α-error accumulation due to multiple testing, the significance level of α = 0.05 was adjusted using a Bonferroni-Holm (BH) (Holm, 1979) correction procedure for the ROIs in each hemisphere, separately. Post hoc analysis was conducted by means of two-tailed t-tests for paired and independent samples.

In order to assess the relationship between cortical activation and behavioural performance, correlations between the number of recited words and CBSI-concentration were calculated at t1 and t2 for each group and task separately by means of Spearman's rho. To further directly consider changes over time, correlations between the differences (t2−t1) in CBSI concentrations and number of recited words were calculated. For post hoc t-tests and correlations, one-tailed P-values were considered in case of directed hypotheses.

2.1.4 Result

2.1.4.1 Sample characteristics

Tables Tables1 and 2 give an overview of the sociodemographic sample characteristics at baseline and clinical questionnaire data for t1 and t2. Sociodemographic data did not differ between groups. For the clinical questionnaire data, no significant differences emerged between the sham- and verum-stimulated group for t1. Verum group versus controls and sham group versus controls, respectively, revealed significant differences on all scales in the expected directions (data shown for HAM-A, self-rated PAS, and CAQ, Table 2).

Table 2: Clinical characteristics of all groups, before and after rTMS treatment.

Group	t1 HAM-A Mean (SD)	t2 HAM-A Mean (SD)	t1 Self-rated PAS Mean (SD)	t2 Self-rated PAS Mean (SD)	t1 CAQ Mean (SD)	t2 CAQ Mean (SD)
Controls	3.83 (3.20) [a, b]	2.74 (3.57) [c, d]	0.22 (1.04) [a, b]	0.13 (0.34) [c, d]	0.33 (0.20) [a, b]	0.33 (0.22) [c, d]
Sham	20.3 (7.10)	15.20 (8.81) [e]	20.52 (8.10)	15.34 (8.30) [e]	1.36 (0.51)	1.06 (0.65) [f]
Verum	22.41 (8.97)	18.37 (10.05) [e]	20.76 (7.76)	14.91 (6.90) [f]	1.63 (0.71)	1.20 (0.71) [f]

Over the course of treatment, the degree of assessed symptoms on HAM-A, self-rated PAS and CAQ declined significantly in the verum and sham stimulated group. However, no significant differences after treatment between these two groups occurred. [a] $p < 0.001$ compared with sham rTMS (t1); [b] $p < 0.001$ compared with verum rTMS (t1); [c] $p < 0.001$ compared with sham rTMS (t2); [d] $p < 0.001$ compared with verum rTMS (t2); [e] $p < 0.01$ t-test for paired samples; [f] $p < 0.001$ t-test for paired samples; CAQ: Cardiac Anxiety Questionnaire; HAM-A: Hamilton Anxiety Rating Scale; PAS: Panic and Agoraphobia Scale; rTMS: repetitive transcanial magnetic stimulation; SD: standard deviation; t1: measuring time 1; t2: measuring time 2.

When patients were asked to guess whether they had received active or sham rTMS, 16 patients in the sham group thought that they had been sham stimulated while 5 thought that it had been the active protocol. Fourteen patients in the verum group thought they had obtained the active protocol and 4 said that they received a placebo treatment. Additionally, 5 patients (1 sham, 4 verum) did not reply to the question. For each patient group, these guesses differed significantly from chance (binomial test, sham group: $P = 0.027$ and verum group: $P = 0.031$).

2.1.4.2 Behavioural performance

Table 3 contains means and standard deviations for the number of produced nouns for the phonological as well as the semantical task for each group and each measuring time.

Table 3: Number of produced nouns for phonological and semantical task for t1 and t2.

	Controls		**Sham**		**Verum**	
Time	Phonological Mean (SD)	Semantical Mean (SD)	Phonological Mean (SD)	Semantical Mean (SD)	Phonological Mean (SD)	Semantical Mean (SD)
t1	20 (7.6)	37.2 (7.2)	18.4 (7.2)	33.2 (7.4)	16.9 (6.4)	34.3 (7.8)
t2	19.7 (7.0)	38.2 (10.1)	19.2 (7.2)	32.5 (7.4)	19.4 (7.8)	35.5 (8.8)

SD: standard deviation; t1: measuring time 1; t2: measuring time 2 after 3 weeks.

With respect to behavioural data, no significant baseline differences could be found between patients and controls. Further the 2 × 3 RM-ANOVA revealed no significant changes for either the phonological or the semantical task.

2.1.4.3 Prefrontal activitiy at baseline

Because one patient missed t2, the fNIRS-data of this subject were excluded from all analyses. Concerning the remaining subjects, significant results were found for all ROIs on both hemispheres for the phonological task (Figure 2) whereby the healthy controls displayed more activation than the patients (left DLPFC: $F_{1,65} = 9.304$, $P = 0.003$, left MSTG: $F_{1,65} = 8.795$, $P = 0.004$, left IFG: $F_{1,65} = 5.279$, $P = 0.025$, right DLPFC: $F_{1,65} = 11.649$, $P = 0.001$, right MSTG: $F_{1,65} = 5.158$, $P = 0.026$, right IFG: $F_{1,65} = 8.130$, $P = 0.006$, all P BH-corrected). For the semantical task significant differences in terms of higher activation in the healthy controls were found only for the DLPFC bilaterally (left DLPFC: $F_{1,65} = 6.189$, $P = 0.015$, right DLPFC: $F_{1,65} = 11.176$, $P = 0.001$, all P BH-corrected). For the control task no significant differences were found (Figure 3).

Figure 2

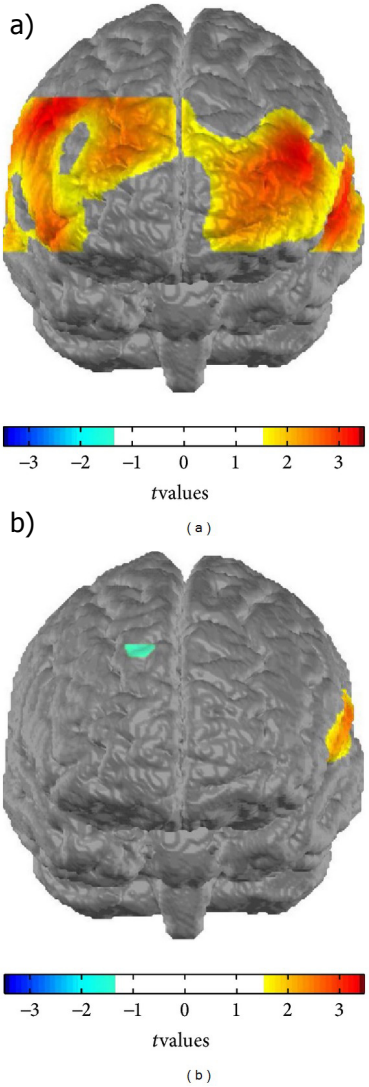


Figure 3

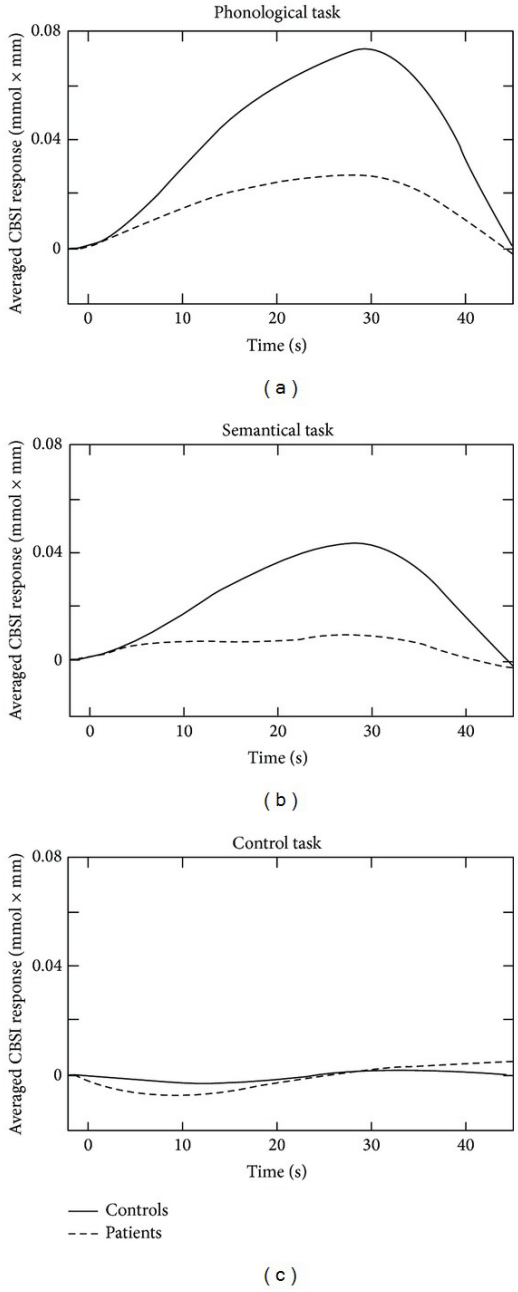


Figure 2: Contrast maps phonological task. Differential CBSI concentration levels contrasted between groups ((a) controls versus PD-patients and (b) verum versus sham) for the phonological task at baseline. Differences in CBSI levels between groups are depicted by means of *t* -values for each channel, whereby only $t \leq 1.7$ values for are shown.

Figure 3: Haemodynamic response function of the left dorsolateral prefrontal cortex at the baseline measurement, averaged over all subjects for each task, separately.

2.1.4.4 Effects of hemispheric lateralisation

Regarding hemispheric lateralisation effects, the 2 × 3 RM-ANOVA showed a significant main effect for the two language related ROIs IFG ($F_{1,65} = 15.030$, $P < 0.001$ (<0.0167, BH-corrected)) and MSTG ($F_{1,65} = 8.317$, $P = 0.005$ (<0.025, BH-corrected)) where activation—as indicated by CBSI concentration—was higher for the left hemisphere. A significant main effect of task was identified for all ROIs (DLPFC: $F_{2,100} = 24.275$ $P < 0.001$ (<0.0167, BH-corrected), MSTG: $F_{2,100} = 55.974$ $P < 0.001$ (<0.025, BH-corrected), and IFG: $F_{2,100} = 61{,}718$ $P < 0.001$ (<0.05, BH-corrected)). The interaction hemisphere∗task was significant for the IFG ($F_{2,130} = 8.151$, $P < 0.001$ (<0.0167, BH-corrected) and the MSTG ($F_{2,114} = 3.478$, $P = 0.040$ (<0.05, BH-corrected)). Post hoc analyses showed that this was due to a left lateralisation concerning the phonological (IFG, right versus left: $t_{65} = -3.734$, $P < 0.001$ and MSTG, right versus left: $t_{65} = -2.983$, $P = 0.002$) and partly the semantical (IFG, right versus left: $t_{65} = -4.034$, $P < 0.001$) task while there was no significant difference for the control task. Regarding the DLPFC, no significant main effect of hemisphere was found, whereas the interaction hemisphere∗task was significant ($F_{2,130} = 11.040$, $P < 0.001$ (<0.025, BH-corrected)). For the DLPFC, results were in contrast to the above-mentioned findings with a significant lateralisation effect in terms of increased activation in the right hemisphere for the control task ($t_{65} = 5.072$, $P < 0.001$) but no significant difference for the two active verbal fluency tasks. Differences between tasks were significant for all comparisons for the IFG (right hemisphere: $t_{65} \geq 2.7$, $P \leq 0.005$ and left hemisphere: $t_{65} \geq 3.37$, $P < 0.001$) and left MSTG ($t_{65} \geq 3.322$, $P < 0.001$) with activation during the phonological task > activation during the semantical task > the control task. For the right hemisphere of the DLPFC, activation during the phonological task was also higher than for the semantical task ($t_{65} = 6.083$, $P < 0.001$). For the left DLPFC, participants showed similar activation patterns as for the IFG and left MSTG with respect to the three test tasks (phonological > semantical > control, for all: $t_{65} \geq 3.114$, $P \leq 0.0015$).

2.1.4.5 Effects of rTMS on prefrontal activity

For the left DLPFC, the analyses of the phonological task showed a significant main effect of group ($F2, 63 = 5.32$, $P = 0.007$ (<0.0167, BH-corrected)). Post hoc analyses revealed that this was due to significantly lower cortical activation of patients in the sham ($t42 = -2.13$, $P = 0.02$) and verum group ($t43 = -2.74$, $P = 0.005$) compared to healthy controls. No significant interaction effect of time and group or main effect of time was found. For the right DLPFC, a significant main effect of group ($F2,63 = 5.34$, $P = 0.007$ (<0.0167, BH-corrected)) was found. No significant effect of time or significant interaction effect of time and group existed with respect to the phonological task. Post hoc *t*-tests displayed similar results as for the left DLPFC. Verum- and sham-stimulated patients showed a reduced activation compared to healthy controls (for both: $t32 \leq -2.348$, $P \leq 0.013$).

For the semantical task, a significant main effect of group was found for the left and the right DLPFC (for both: $F2,63 \geq 5.30$, $P \leq 0.007$ (<0.0167, BH-corrected)). For both areas, actively stimulated patients showed a significantly reduced cortical activation compared to healthy controls (left DLPFC: $t35 = -2.78$, $P = 0.005$ and right DLPFC: $t43 = -2.60$, $P = 0.007$). Also, sham-stimulated patients showed significant hypoactivation compared to healthy participants with respect to the right ($t38 = -3.19$, $P = 0.002$) and left DLPFC ($t34 = -2.316$, $P = 0.014$). No significant main effects of time or significant interactions of time and group were discerned for the left and right DLPFC, respectively. No significant differences between sham- and verum-stimulated patients existed with regard to the left or right DLPFC for the phonological and semantical task, respectively.

The analyses of the control task for the left and right DLPFC revealed neither significant main effects of group nor significant main effects of time. Also, no significant interaction effects of time and group were found.

For reasons of clarity, solely significant results for the IFG with respect to the three test tasks are depicted in Table 4. For the MSTG, no significant outcomes were found.

Table 4: Significant results for the cognitive tasks with respect to the IFG.

ROI	df (df error)	F	p	Verum vs. Sham	Verum vs. Controls	Sham vs. Controls	Paired t-tests
Left IFG – phonological task							
Time x Group	2 (63)	5.23	0.008 (< 0.0167, BH-corrected)	t1: ns. t2: ns.	t1: ns. t2: ns.	t1: S < HC** t2: ns.	S: t1 < t2* V: ns. HC: ns.

*significant at a significance level of ≤0.05, **significant at a significance level of ≤0.01, BH-corrected: Bonferroni-Holm-corrected, HC: healthy controls, IFG: inferior frontal gyrus, S: sham group, and V: verum group.

2.1.4.6 Correlations between fNIRS data and behavioural performance

At baseline, no significant correlations between CBSI concentration and the number of recited words were found for either PD-patients or for the healthy controls. At the second measurement time, a relationship was merely found for the healthy controls in terms of negative correlations for all ROIs, except for the right DLPFC with the number of recited words during the phonological task (left DLPFC: $r = -0.416$, $P = 0.024$, left MSTG: $r = -0.431$, $P = 0.020$, left IFG: $r = -0.452$, $P = 0.015$, right MSTG: $r = -0.534$, $P = 0.004$, right IFG: $r = -0.558$, $P = 0.003$, all P BH-corrected). Regarding changes over time, significant results existed only during the phonological task in the two patients' groups. In this context, an increase in the number of recited words was significantly associated with a decrease in CBSI concentration (resp., vice versa) for the DLPFC (sham, left DLPFC: $r = -0.498$, $P = 0.011$, verum, left DLPFC: $r = -0.485$, $P = 0.011$, verum, right DLPFC: $r = -0.607$, $P = 0.001$, all P BH-corrected). As all correlations were negative, they were only considered explorative, as positive correlations were hypothesized and one-sided tests were conducted.

2.1.5 Discussion

The present study aimed to confirm the finding that PD-patients are characterised by prefrontal hypoactivation during cognitive tasks as compared to healthy controls

(Ohta et al., 2008). Moreover, it additionally addressed the question whether a potential hypoactivation of the PFC can be normalised by means of repeated iTBS. Patients with PD were investigated via fNIRS while performing a VFT prior to and after receiving daily prefrontal iTBS application over a time course of three weeks in addition to weekly group sessions of psychoeducation. The VFT-results were compared with those of healthy control subjects.

Regarding our first hypothesis, our results are in line with the above-mentioned findings concerning hypofrontality during cognitive tasks in PD-patients. With respect to our second hypothesis, unexpectedly, an increase in activation over time could only be found for the left IFG in sham-stimulated patients.

In more detail, before the start of rTMS treatment, differences in cortical activation (as indicated by CBSI data) between patients and controls were observed for specific task conditions of the VFT. In fact, as predicted by our hypothesis, patients did not differ from controls during the control task but displayed decreased prefrontal activation in all ROIs during the phonological task and partly also during the semantical task. The missing differences during the control task indicate that the differences in CBSI concentration between healthy controls and patients during the two active tasks were indeed due to altered cognitive processing and not to more general effects elicited by the measurement situation. Still, it cannot be excluded that our fNIRS signal may have been affected by components that are not directly related to cognitive processing but still lead to a (task-related) change in blood flow and hence a change of the measured signal. Regarding more general effects that might influence the fNIRS signal, a recent study by Takahashi et al. (2011) showed that the verbal fluency task is particularly affected by confounding effects due to stress induced skin blood flow, especially for NIRS channels located over the forehead. In order to verify that we still mainly measured cortical activation, we presumed that lateralisation effects in terms of increased left hemispheric activation should be found for language related areas such as the MSTG and IFG but not for the DLPFC. Further, increases in these two ROIs should only exist for the semantical and phonological but not for the control task. In line with previous studies (Tupak et al., 2012) we could confirm these assumptions and accordingly ascribe our finding mainly to differences in cortical activation.

Contrary to our second hypothesis, no significant changes in prefrontal activation after rTMS treatment could be found in the verum group. In fact, the only significant change was found for the sham group which showed an increase in CBSI concentration in the left IFG during the phonological task. As at first glance these findings are hard to interpret and we further analysed the prefrontal activation patterns in relation to the behavioural performance of healthy controls and the two patients groups.

When regarding only the behavioural data, descriptively, healthy controls could name more nouns than both patients groups; however, this difference was not significant. Further, when associating CBSI concentrations in the different ROIs with the number of recited nouns at baseline, no significant correlations could be revealed for either group. Interestingly, however, at the second measurement time, negative correlations between the behavioural performance and activation patterns in nearly all ROIs existed for the healthy controls. Even though we originally applied one-sided testing (assuming a positive relationship between behavioural performance and cortical activation), we still think that it is worthwhile to give these negative correlations some considerations as they might be helpful for a better understanding of our results.

Similar to the finding in healthy controls, negative associations between changes in the number of recited nouns from *t*1 to *t*2 and changes in DLPFC activation bilaterally during the phonological task could be found for both patients groups. In order to interpret these results in a meaningful way, it has to be considered that multiple distinct mechanisms might have an influence on the fNIRS signal. Firstly, according to our hypothesis, it can be assumed that a demanding cognitive task leads to an increase in cortical activation which then triggers a certain performance at the behavioural level. In this context, higher cortical activation should lead to a better behavioural performance as it implies that more cognitive resources can be recruited to fulfil the task as well as possible. From another perspective, one could also assume that in subjects with a highly efficient cortical processing (i.e., in case of a subjectively nonchallenging task situation) fewer cognitive resources are needed to achieve good results. In this case, low cortical activation should be associated with high behavioural performance. However, it needs to be kept in mind that the fNIRS

signal might not just contain components which are due to cortical activation but might also be influenced by extracranial signal components that relate to peripheral processes such as psychophysiological arousal induced changes in blood flow. In particular, in frontopolar regions, these components have been shown to also trigger an increase in the fNIRS signal due to stress induced vasodilation during a verbal fluency task (Haeussinger et al., 2014). In this context, higher CBSI concentrations might then also be associated with a decrease in behavioural performance as it can be presumed that too much psychophysiological arousal should have a negative effect on cognitive functioning. Even though we tried to control for such arousal effects by performing a control task and considering lateralisation effects, we cannot exclude the fact that it still had an effect on our results.

Accordingly, we conclude that we could not find any significant correlations at the baseline measurement time as psychophysiological arousal was probably very high for all participants, hence having confounding effects on the fNIRS signal components due to cortical activation. At the second measurement time, cortical activation should have been the same for the healthy controls while arousal may have decreased for some participants as the situation was more familiar, leading to a reduction in signal intensity and negative correlations with behavioural performance due to improved cognitive function (with reduced arousal). While it cannot be excluded that these negative correlations also imply that the task was not challenging enough for some of the healthy subjects, the study by Takahashi et al. (2011) points more in favour of an interpretation in terms of a decrease in psychophysiological arousal. In fact, the authors could show that already a repetition of the verbal fluency task within one measurement could lead to a significant repetition effect by means of a decrease in psychophysiological arousal and associated fNIRS signal intensity.

Concerning the PD-patients, psychophysiological arousal should have also decreased but possibly not as much as in the healthy controls as the measurement situation still represented a typical panic-relevant situation (patients had to sit in a small room with the fNIRS probe set attached to their heads so a sudden escape was not possible). At the same time it can be expected that arousal effects, which are prominent in the frontopolar area of the PFC, also have an effect especially on the

DLPFC which cannot be neglected (Haeussinger et al., 2014). A possible explanation especially for the influence of DLPFC activation through the frontopolar region is given by Kirilina et al. (2012) who found that the vein responsible for arousal effects in the forehead also stretches out to dorsolateral regions. Consequently, apparent effects of a slight decrease in arousal would most likely be expected in the DLPFC, hence explaining the negative correlations between changes in behavioural performance and changes in CBSI concentrations for the patients. Even though correlations between CBSI concentrations and behavioural performance during the semantical task were not significant, it is noteworthy to mention that the direction of the correlations was generally the same, supporting our prior assumptions.

We therefore conclude that healthy controls as well as patients in both groups were generally less affected by psychophysiological arousal during the second measurement time. In this regard, the increase in activation from the first to the second measurement time for the left IFG in the sham group might not be related to an increase in cognitive functioning but might merely represents a more general possibly also arousal related effect. A further reason which might have contributed to the increase in CBSI concentrations after sham iTBS might be given by simple regression towards the mean. In this regard it needs to be considered that sham- and verum-stimulated patients did not differ significantly in their activation patterns after rTMS application. Instead, sham-stimulated patients showed a significantly decreased baseline CBSI concentration in the left IFG compared to healthy controls. All in all, our findings confirm our first hypothesis that PD-patients show a prefrontal dysfunction that is at least partly independent of panic-related tasks. However, an increase in cortical activation after verum iTBS was not found. Instead, we could accentuate the need to consider task-related arousal induced effects especially when investigating patients with anxiety disorders.

To our knowledge, this is the first controlled study investigating effects of add-on theta burst stimulation (TBS) on prefrontal activation and cognitive functioning in patients with PD/agoraphobia. So far, only a few open studies investigated the effects of TBS on psychiatric symptoms (e.g., Chistyakov, Rubicsek, Kaplan, Zaaroor, & Klein, 2010; Holzer & Padberg, 2010).

However, limitations of this study have to be mentioned. The stimulation condition (verum versus sham) was correctly identified by the majority of patients, so one could argue that placebo effects might have affected our results. Possibly, patients exchanged their perceptions about rTMS during the psychotherapy group sessions, as they became acquainted with each other over the course of psychoeducation. For further investigations, we therefore emphasise the need for specialised sham coils which produce a superficial electrical current on the skull, as demonstrated by Rossi et al. (2007). Although in our study sufficient blinding could not be reached, promising results of rTMS in controlled studies with electromagnetic placebo coils could demonstrate specific effects of verum stimulation on psychiatric symptoms (e.g., for PTSD and comorbid depression by Boggio et al. (2010). Referring to the choice of the rTMS-frequency, we used a protocol which is assumed to facilitate motor cortex excitability (Huang et al., 2005). Also, a facilitation of frontal activity could be demonstrated. For example, speech repetition accuracy was promoted by intermittent theta burst stimulation of the left posterior inferior frontal gyrus (Restle et al., 2012). Nevertheless, rTMS effects seem to be influenced by a wide range of factors, for example, genetic variables or the way of application. Cheeran et al. (2008) could demonstrate a significant influence of the brain-derived neurotrophic factor gene (BDNF) on the TBS-efficacy for the primary motor cortex. Also, TBS after-effects seem to hinge on the NMDA-receptor (Huang, Chen, Rothwell, & Wen, 2007). Further, a study of Gamboa, Antal, Moliadze, & Paulus (2010) demonstrated reversed iTBS-effects after a prolonged, single application of 1200 instead of 600 stimuli. Taken together, it could be questionable if iTBS consistently facilitates the excitability of stimulated neurons. Moreover, in our study, rTMS was generally applied after psychoeducation sessions. However, an application prior to psychoeducation could have led to a different processing of the afterwards presented information. We therefore suggest that future studies should systematically assess temporal effects of rTMS applications in relation to additional intervention methods. Regarding methodology, we have already discussed the problems that arise from the confounding skin blood flow signal component in the fNIRS data. A possible solution to this—which allows for an even more precise interpretation of the result—might be to measure the skin components selectively by additionally placing optodes with

shorter interoptode distances on the probe set (Takahashi et al., 2011). Finally, concerning the diagnostic process, PD/agoraphobia was diagnosed prior to *t*1 with the help of structured clinical interviews. However, the time lag between these interviews and *t*1 was not standardized in our study.

2.1.6 Conclusion

This pilot study investigated cortical activation patterns of patients with PD/agoraphobia compared to healthy controls. Further, effects of add-on iTBS on cortical activation and cognitive performance in PD/agoraphobia were analysed. Findings of a baseline cortical hypoactivation could be replicated. However, an increase in cortical activation after verum iTBS could not be supported. Instead we only found increased CBSI concentrations for the left IFG after sham iTBS application. By integrating behavioural performance into our analysis we could attribute this finding to more general effects such as task-related psychophysiological arousal and regression towards the mean. Taken together, our results confirm that PD is characterised by prefrontal hypoactivation. As we could not verify an increase in cortical activation after verum iTBS, further studies that should control for task-related psychophysiological arousal are needed in order to evaluate under which circumstances iTBS might serve as a therapeutic tool in the treatment of PD.

2.2 Manuscript 2: Neurobiological and clinical effects of fNIRS-controlled rTMS in patients with panic disorder/agoraphobia during cognitive-behavioural therapy

The contents of this chapter are published in:
Deppermann, S.*, Vennewald, N.*, Diemer, J., Sickinger, S., Haeussinger, F.B., Dresler, T., Notzon,S., Laeger,I., Arolt,V., Ehlis, A.-C., Fallgatter, A.J., Zwanzger, P. (2017). Neurobiological and clinical effects of fNIRS-controlled rTMS in patients with panic disorder/agoraphobia during cognitive-behavioural therapy.
NeuroImage: Clinical, 16:668, 2017.

* Both authors contributed equally to this work

2.2.1 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.

2.2.2 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 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 hypoactivation 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-situal 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.

2.2.3 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.

2.2.3.1 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.

Table 1. Baseline sample characteristics.

	Verum	Sham	Controls	Statistics	Post-hoc
Number in sample	22 (14)	22 (12)	23 (19)		
Mean age in years (range)	37.6 (19-63) (38.4 (21-63))	36.3 (22-56) (39.1 (24-56)	33.4 (19-64) (34.7 (22-64))	$F_{2,66} = 0.807$, $p = 0.45$ ($F_{2,44} = 0.74$, $p = 0.48$)	
% women	59 (50)	64 (75)	61 (63)	$X^2 = 0.097$, $p = 0.95$ ($z = 1.70$, $p = 0.43$)	
Handedness (number of right-handed subjects)	20 (13)	21 (12)	20 (16)	$z = 1.037$, $p = 0.87$ ($z = 1.89$, $p = 0.45$)	
First Language	19 (13) german 1 (0) bilingual 2 (1) other	19 (11) german 2 (1) bilingual 1 (0) other	22 (18) german 1(1) bilingual -	$z = 2.74$, $p = 0.64$ ($z = 5.73$, $p = 0.50$)	
Mean years of education (SD)	12.1 (1.7) (12.2 (1.8))	12.4 (2.0) (12.3 (2.4))	12.5 (1.1) (12.4 (1.2))	$F_{2,66} = 0.33$, $p = 0.72$ ($F_{2,44} = 0.033$, $p = 0.97$)	
Mean duration of illness in months (range)	92 (1-372) (109.8 (18.372))	84 (1-336) (111.2 (5-336))	-	$F_{1,43} = 0.084$, $p = 0.77$ ($F_{1,25} = 0.001$, $p = 0.97$)	
Comorbid depression	8 (4) currently 9 (7) in past 5 (3) never	6 (2) currently 11 (8) in past 5 (2) never	-	$z = 0.56$, $p = 0.92$ ($z = 0.86$, $p = 0.76$)	
Mean HAM-A – total (SD)	22.41 (8.97) (21.14 (8.01))	20.3 (7.1) (20.25 (8.66))	3.90 (3.35) (0.26 (1.15))	$F_{2,66} = 50.49$, $p < 0.001$ ($F_{2,44} = 33.45$, $p < 0.001$)	V = S > HC (V=S > HC)
Mean self-rated PAS total (SD)	20.76 (7.76) (18.02 (7.92))	20.52 (8.10) (18.83 (9.43))	0.22 (1.04) (4.37 (3.24))	$F_{2,66} = 75.64$, $p < 0.001$ ($F_{2,44} = 41.75$, $p < 0.001$)	V = S > HC (V=S > HC)
Mean CAQ – total (SD)	1.63 (0.71) (1.52 (0.67))	1.36 (0.51) (1.36 (0.54))	0.33 (0.20) (0.32 (0.22))	$F_{2,66} = 39.95$, $p < 0.001$ ($F_{2,44} = 29.49$, $p < 0.001$)	V = S > HC (V=S > HC)

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].

2.2.3.2 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).

Figure 1

<table>
<tr><td>Screening</td><td colspan="3">Screening patients: n = 312 / eligable to participate n = 72 / participation after informed consent: n = 63 / drop-outs: n = 19 (thereof n = 12 prior to treatment start; n = 6 during treatment)</td></tr>
<tr><td></td><td colspan="2">Patients</td><td>Healthy controls</td></tr>
<tr><td>Baseline</td><td colspan="2">Randomization
fNIRS / HAM-A / PAS / CAQ (baseline)</td><td>fNIRS / HAM-A / PAS / CAQ (baseline)</td></tr>
<tr><td>iTBS-7 (day 7)
iTBS-14 (day 14)</td><td>HAM-A / PAS / CAQ
HAM-A / PAS / CAQ</td><td>CBT
• S1: Psychoeducation
• S2: Psychoeducation
• S3: Psychoeducation</td><td>ITBS
Active or sham stimulation</td></tr>
<tr><td>Post-iTBS (day 21)</td><td>fNIRS / HAM-A / PAS / CAQ</td><td>• S4: Introduction exposure session</td><td>fNIRS / HAM-A / PAS / CAQ</td></tr>
<tr><td>Post-CBT</td><td>HAM-A / PAS / CAQ</td><td>• S5: Preparing exposure session
• S6: Exposure session (single – setting)
• S7: Cognitive aspects of the vicious cycle
• S8: Stress management
• S9: Summary & relapse prevention</td><td></td></tr>
<tr><td>Follow-up 1</td><td colspan="2">HAM-A / PAS / CAQ (3 month) / booster session CBT</td><td></td></tr>
<tr><td>Follow-up 2</td><td colspan="2">HAM-A / PAS / CAQ (6 month) / booster session CBT</td><td></td></tr>
</table>

Study design.

Abbreviations: CAQ, Cardiac anxiety questionnaire; CBT, Cognitive Behavioural Therapy; fNIRS, functional near-infrared spectroscopy; HAM-A, Hamilton Anxiety Rating Scale; iTBS, intermittent Theta Burst Stimulation; PAS, Panic and Agoraphobia Scale; S1-S9, therapy sessions 1 to 9.

2.2.3.3 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).

2.2.3.4 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-B65, 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 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.

2.2.3.5 Outcome measures

2.2.3.5.1 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 differ significantly 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.

2.2.3.5.2 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 photo-detectors 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 O2Hb 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).

2.2.3.5.3 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.

2.2.3.6 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

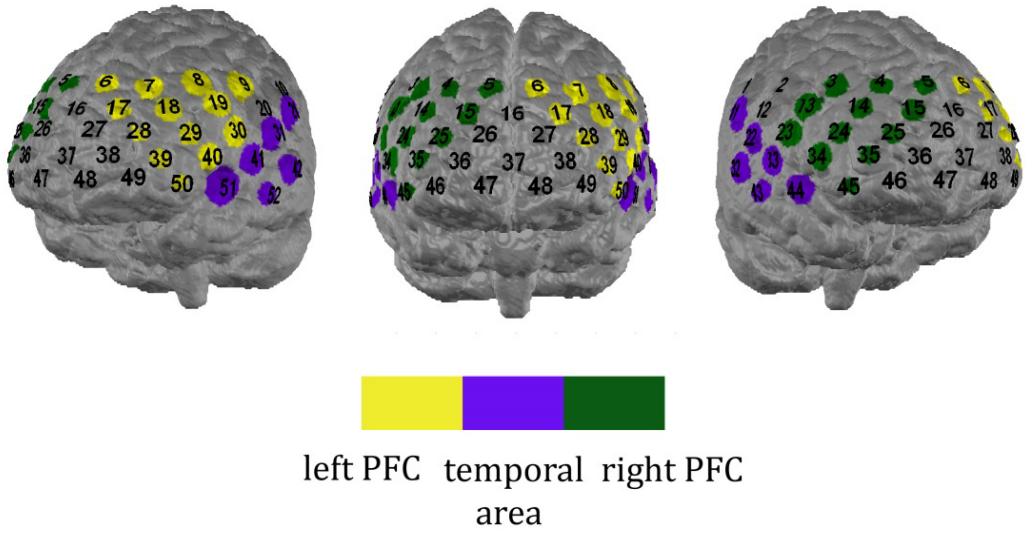
sets were only available from $n = 20$ verum-stimulated patients, $n = 21$ sham-stimulated patients, and $n = 21$ healthy controls. The data of the remaining participants were segmented channel-wise in an event-related manner. A time frame of 0–16 s after stimulus onset was extracted and adjusted for 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.

2.2.3.7 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.

Figure 2



Probe set arrangement with numbers indicating channels.

PFC: prefrontal cortex; colour-coded channels were used for analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2.3.8 Statistical analyses

Baseline sample characteristics were tested with one-way ANOVAs, χ2- or *t*-tests, depending on the variable in question. Fisher's exact test was used for analysing baseline sample characteristics if there were fewer than five cases per category.

To evaluate the effectiveness of patient blinding regarding the iTBS treatment condition, we conducted binomial tests of the subjectively perceived iTBS condition (test proportion: 0.5) for each group.

Regarding fNIRS data, for both ROIs, 2 × 2 × 3 repeated measurement analyses of variance (RM-ANOVAs) were conducted with the within-subject factors condition (panic-related vs. neutral words) and time (pre vs. post iTBS treatment) and the between-subject factor group (verum vs. sham vs. controls). An RM-ANOVA was performed for the temporal "non-ROI".

Behavioural data (RTs and ERs) were analysed by means of RM-ANOVAs.

For the clinical data, 2 × 3 RM-ANOVAs were conducted with the within-subject factor time (baseline vs. post-iTBS) and the between-subject factor group (verum vs. sham vs. controls) considering differences between the three groups on the total scores (CAQ, HAM-A, PAS self-rated). The content of the subscales of all questionnaires was grouped according to the topics (as outlined in the Supplementary material) and Bonferroni-Holm-correction (Holm, 1979) was applied within each topic.

To analyse the course of iTBS effects on clinical data over time, RM-ANOVAs (7 × 2-design) were calculated with the within-subject factor time (from baseline to follow-up 2) and the between-subject factor group (verum vs. sham). The following post-hoc comparisons were conducted: baseline vs. post-iTBS, baseline vs. post-CBT, baseline vs. follow up 1, baseline vs. follow-up 2, follow-up 1 vs. follow-up 2, post-iTBS vs. post-CBT, post-iTBS vs. follow-up 1, and post-iTBS vs. follow-up 2 Two-tailed *t*-tests for matched samples were employed for post-hoc analyses.

Correlations (Spearman's rho) between the CBSI concentrations and the questionnaire subscales were calculated for the sham and verum group at baseline and post-iTBS. To do so, the difference between activation elicited by panic-related and neutral words was calculated. Changes in these CBSI concentrations (CBSIpost-iTBS - CBSIbaseline) were correlated with changes in the questionnaire scores (post-iTBS - baseline).

Behavioural data (RTs and ERs) were available from n = 46 participants (20 controls, 14 verum, 12 sham patients). Due to technical problems, button presses were not recorded properly for the remaining participants and one control subject had to be excluded due a too high ER (> 2 standard deviations). Again, it was verified that groups did not differ significantly concerning baseline characteristics (Table 1).

2.2.4 Results

2.2 4.1 Sample Characteristics

Table 1 shows the sample characteristics for the verum and sham groups as well as the healthy controls. No significant group differences for sociodemographic variables were found for the complete sample or the sub-sample (values in brackets) used for the analysis of the behavioural data. For clinical ratings, no significant differences existed between verum and sham group. Compared to the control group, clinical ratings were significantly higher for both patient groups (Table 1).

2.2.4.2 Manipulation check

2.2.4.2.1 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).

2.2.4.2.2 Emotional Stroop task - behavioural data

For the behavioural 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 ($F_{1,42} = 4.622$, $p = 0.037$) as well as ERs ($F_{1,42} = 5.6$, $p = 0.007$). Furthermore, a significant main effect for the factor condition ($F_{1\ 42} = 180, 109$, $p < 0.001$) and the factor group ($F_{2,42} = 2.42$, $p = 0.04$) was detected for ERs only. As can be seen in Table 2, all subjects committed more errors for panic-related words then for neutral words but the sham-stimulated patients generally committed the fewest errors (verum vs. sham: $t_{24} = 2.098$, $p = 0.047$; controls vs. sham: $t_{29} = 2.958$, $p = 0.006$). There were no significant interactions. Mean RTs and ERs are for all groups, times and conditions are shown in Table 2.

Table 2. Mean and Standard Deviation of reaction times (RT) and error rates (ER

		Sham	Verum	Controls
RTs/ ERs				
Panic-related	**Baseline**	772 (122) 3.8 (0.8)	800 (80) 4.0 (1.0)	765 (116) 4.2 (1.5)
	Post-iTBS	808 (110) 4.1 (1.4)	812 (90) 4.6 (1.7)	800 (102) 5.4 (1.6)
neutral	**Baseline**	771 (111) 0.5 (0.8)	799 (80) 2.0 (1.6)	769 (117) 1.8 (1.4)
	Post-iTBS	802 (124) 1.4 (1.0)	813 (96) 1.7 (1.5)	790 (106) 1.9 (1.7)

ms, milliseconds; iTBS, intermittent theta burst stimulation

2.2.4.3 fNIRS Data - baseline differences and treatment effects

The 2 × 2 × 3 RM-ANOVAs of CBSI concentrations revealed no significant main effects, but a significant three- way interaction of condition ∗ time ∗ group for both the left ($F_{2,59} = 4.017$, $p = 0.023$) and right PFC ($F_{2,59} = 3.836$, $p = 0.027$).

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 (panic vs. neutral): $t19 = -2.126$, $p = 0.047$), 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 (t39 = 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 (t39 = − 2.052, $p = 0.047$) and more activation for neutral words (t39 = 2.528, $p = 0.016$) in the control group compared to the verum group. The same pattern emerged when contrasting the sham and verum group: verum-stimulated patients showed more activation for panic-related words (t39 = − 2.054, $p = 0.047$) and less activation for neutral words (t39 = 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.

Figure 3

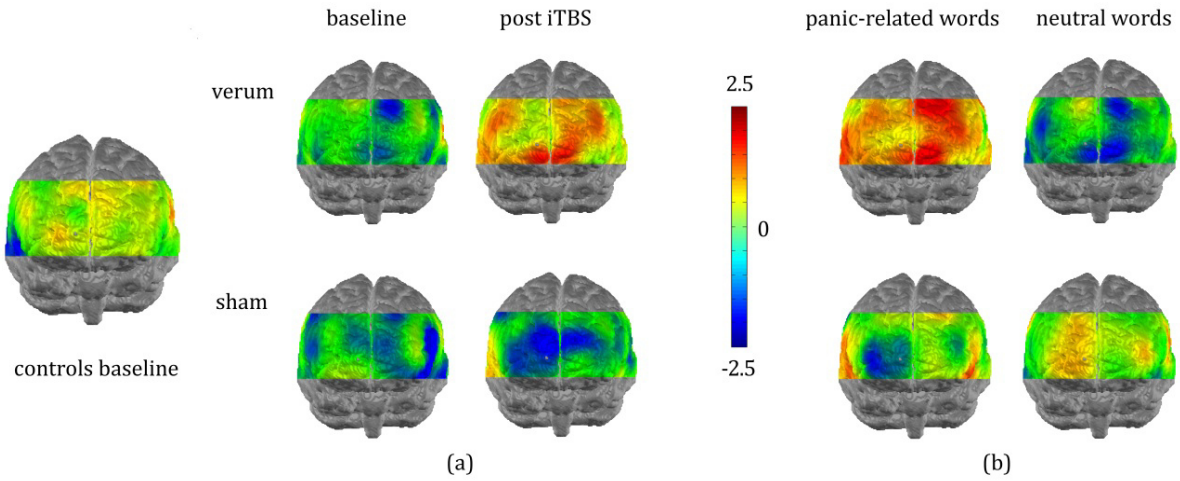


Figure 3a: Contrast maps panic-related words vs. neutral words for each group.

Figure 3a depicts differential CBSI concentration levels contrasted between the two conditions (panic-related words vs. neutral words) by means of t-values for each channel. (Intended for colour reproduction)

Fig. 3b: Contrast maps iTBS-related activation changes.

Figure 3b illustrates the changes in CBSI concentration levels from baseline to post-iTBSin the two patients groups by means of t-values for each channel, whereby positive values indicate an increase and negative a decrease in activation.

2.2.4.4 Clinical data

For the total scores (PAS-total, HAM-A total, CAQ-total), 2 × 3 RM-ANOVAs revealed significant main effects for the factors time and group, as well as a significant time × 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.

Table 3. Clinical course of agoraphobic avoidance behaviour from baseline to follow-up 2.

		Verum (n= 22)	**Sham (n=22)**	**F_{df}, p**	**Post hoc tests**		
	Measurement time	Mean (SD)	Mean (SD)	time time x group	patient group - total	verum group	sham group
PAS (OR) Agoraphobic avoidance	Baseline	1.91 (1.22)	1.39 (1.19)	$F_{6,252}$ = 7.91, < 0.001	Baseline > post- iTBS * post-CBT***, follow-up1*** follow-up2***		
	iTBS-7	1.35 (1.14)	0.96 (1.04)				
	iTBS-14	1.14 (1.23)	1.00 (1.03)	ns.			
	post-iTBS	1.20 (1.08)	1.20 (1.24)		post- iTBS > post-CBT* follow-up 1*** follow-up 2**		
	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		
PAS (SR) Agoraphobic avoidance	Baseline	2.03 (1.02)	1.80 (1.10)	$F_{4, 179}$ = 9.6, < 0.001			
	iTBS -7	2.22 (1.01)	1.21 (0.91)	$F_{4,179}$ = 3.39, = 0.009			
	iTBS -14	1.97 (0.87)	1.58 (1.04)				
	post- iTBS	1.74 (0.70)	1.50 (1.14)				
	post-CBT	1.54 (0.82)	1.11 (0.98)			Baseline > post-CBT* follow-up1*** follow-up2**	Baseline > post-CBT** follow-up1*** follow-up2*
	Follow- up 1	1.18 (0.91)	1.08 (0.86)			post- iTBS = post CBT post- iTBS > follow-up 1** follow-up 2*	post- iTBS = post-CBT follow-up 1 follow-up 2
	Follow-up 2	1.29 (0.89)	1.35 (0.80)			follow-up 1 = follow–up 2	follow-up 1 < follow–up 2*

*, 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; CBT, cognitive behavioural therapy; df, degrees of freedom; F, F-value; ns.; iTBS, intermittent Theta Burst Stimulation; 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.

2.2.4.5 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.

2.2.5 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 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 hypoactivation 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.

2.2.6 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 (Osuch 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.

2.2.7 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.

3. Study 2: Clinical and neurobiological effects of fNIRS-controlled transcranial magnetic stimulation (rTMS) in patients with spider phobia

3.1 Manuscript 1: Psychophysiological effects of an rTMS modulated virtual reality challenge including participants with spider phobia

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3.1.1 Abstract

Preliminary evidence suggests beneficial effects of transcranial magnetic stimulation (TMS) on anxiety. The objective of this study was to investigate the effects of intermittent theta burst stimulation (iTBS) as a form of TMS on acute anxiety provoked by a virtual reality (VR) scenario.

Participants with spider phobia (n = 41) and healthy controls (n = 42) were exposed to a spider scenario in VR after one session of iTBS over the prefrontal cortex or sham treatment.

Participants with spider phobia reacted with more anxiety compared to healthy controls. Their heart rate and skin conductance increased compared to baseline. Contrary to expectations, iTBS did not influence these reactions, but modulated heart rate variability (HRV). Sympathetic influence on HRV showed an increase in the active iTBS group only. This study does not support the idea of beneficial effects of a single session of iTBS on anxiety, although other protocols or repeated sessions might be effective.

3.1.2 Introduction

Converging evidence from many studies suggests that raised activity of the amygdala plays a key role in the development of fear and anxiety. According to this model, pathological anxiety is the result of inadequate amygdala activation to non-threatening stimuli. Since the prefrontal cortex (PFC) has an inhibitory effect on the amygdala, this hyperactivity is attributed to a dysfunction of the PFC which results in an insufficient suppression of the amygdala (Eden et al., 2015; Etkin & Wager, 2007; Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003). For example, Nishimura et al. (2007) reported hypoactivation of the left PFC in patients with panic disorder. Regarding spider phobia, Johanson, Risberg, Tucker, & Gustafson (2006) found an increase in bilateral prefrontal cerebral blood flow in initially strongly anxious patients with spider phobia after successful cognitive psychotherapy. Within the PFC the dorsolateral prefrontal cortex (DLPFC) has been shown to play a role in the processing of information with emotional content (Dolcos, LaBar, & Cabeza, 2004; Meyer-Lindenberg et al., 2005). However, other regions within the PFC like the dorsomedial prefrontal cortex may play an equally or even more important role in the interaction with the amygdala regarding anxiety (Robinson, Charney, Overstreet, Vytal, & Grillon, 2012; Robinson et al., 2014).

Such a model of a dysbalance regarding the interaction of PFC and amygdala constitutes the use of transcranial magnetic stimulation (TMS) to modulate cortical activity non-invasively and thus influences the function of the PFC (Diemer et al., 2010; Pallanti & Bernardi, 2009). Despite a paucity of evidence at a neural network-level for the capability of prefrontal cortical TMS to influence the activity of the amygdala, TMS has been investigated in a repetitive form (rTMS) as a potential therapeutic intervention in depression (Herwig et al., 2007; Padberg et al., 1999; Pallanti & Bernardi, 2009; Plewnia et al., 2014) and panic disorder (see section 2; Dresler et al., 2009; Zwanzger et al., 2009; Zwanzger et al., 2002) aiming at an increase of PFC function. Intermittent theta burst stimulation (iTBS) is a more intense, innovative form of TMS that comprises the repeated application of bursts of stimuli and facilitates excitation in cortical circuits (Huang et al., 2005). In addition to the above described model of prefrontal top-down control, the "valence hypothesis" is another neurobiological model which has often been used to explain the

pathogenesis of anxiety disorders and depression (Vennewald et al., 2013). Accordingly, approach related emotions are rather modulated in the left hemisphere, while avoidance related emotions are rather modulated in the right hemisphere. In line with this idea, the most widely studied forms of TMS in major depression and anxiety disorders are low frequency rTMS over the right DLPFC and high frequency rTMS over the left DLPFC (Lefaucheur et al., 2014). At least in the context of major depression, a review by Chen et al. (2013) came to the conclusion that both stimulation protocols are equally effective. Since other interventions (e.g., antidepressant medication) have been demonstrated to be effective in both psychiatric disorders, its successful use in the treatment of depression makes TMS a promising therapeutic option in anxiety disorders. As there is not enough evidence which suggests to favor one over the other stimulation technique (Lefaucheur et al., 2014), we decided to investigate iTBS over the left DLPFC which is comparable to high frequency rTMS (Huang et al., 2005), since this suits the model of prefrontal top–down control as well as the "valence hypothesis". However, it should be kept in mind that these hypotheses simplify both, the underlying neural network as well as the mode of action of TMS, which are not fully understood yet and are for sure more complex and involve more brain regions than just the PFC and amygdala. For example, Chervyakov, Chernyavsky, Sinitsyn, & Piradov (2015) reviewed the literature about putative and established mechanisms explaining the therapeutic effects of TMS. They point out that TMS does not just induce the transmission of electrical signals to neurons, but also affects neurotransmitters, gene expression, the activity of certain enzymes, cerebral blood flow and many other processes within the brain.

The present study investigated the effect of iTBS on acute anxiety. As a model, specific spider phobia was chosen because the disorder is very common (Fredrikson, Annas, Fischer, & Wik, 1996) and anxiety can be triggered easily by presentation of spiders. For a standardized presentation we chose virtual reality (VR), a technology that permits a very realistic presentation of virtual spiders in three-dimensional scenarios by means of a head-mounted display. VR scenarios are appropriate not only to provoke subjective anxiety, but also psychophysiological changes. A review by Diemer et al. (2014) compared thirty-eight studies on psychophysiological effects

of VR in patients with anxiety disorders as well as healthy participants with and without increased trait anxiety. They found that challenging situations in VR are capable of altering skin conductance levels (SCL) in patients with anxiety disorders as well as in healthy controls. Results for heart rate (HR) are inconclusive.

Since patients experience an increase of SCL and often also HR in a VR exposure scenario, it is important to further investigate whether these parameters decrease with habituation to the scenario, and how long this takes. Only few studies have so far addressed these questions. Patients with fear of flying, for example, have shown reductions in HR and SCL response to virtual flight environments after repeated exposures (Mühlberger et al., 2001). Heart rate variability (HRV) is another parameter of psychophysiological arousal that provides information about influences of the autonomous nervous system on the heart. Two important sub-measures of HRV are LF, the low frequency component, mediated by the sympathetic as well as parasympathetic nervous system, and HF, mediated mainly by the parasympathetic nervous system. High LF as well as low HF point to more sympathetic and less parasympathetic influence, while low LF and high HF are associated with less sympathetic and more parasympathetic influence (Berntson et al., 1997). HRV has rarely been studied in the context of TMS. To the best of our knowledge, there is only one study on the long term effect of rTMS over the prefrontal cortex that measured HRV. Udupa et al. observed a decrease in LF/HF ratio in 30 patients with major depression after 12 sessions of high-frequency (15 Hz) rTMS over the left prefrontal cortex (Udupa et al., 2007; Udupa et al., 2011). As for immediate effects, there have only been studies on regions other than the DLPFC, e.g., Yoshida et al. (2001) found significantly elevated LF as well as HF after low frequency (0.2 Hz) rTMS over the vertex. In contrast to them, Vernieri et al. (2014) found a decrease of LF/HF ratio after low frequency (1 Hz) rTMS over the primary motor cortex.

Closely related to emotions like anxiety and parameters of psychophysiological arousal like HR, SCL and HRV is the feeling of presence in virtual reality. Presence is defined as the impression of really being there in a certain environment, even if it is virtual (Slater, 1999). Strong emotions and arousal have repeatedly been shown to be associated with an increased feeling of presence (Diemer, Alpers, Peperkorn, Shiban, & Mühlberger, 2015).

In the present bicentric study, iTBS was combined with a VR challenge to provoke anxiety in participants with spider phobia in a single-blind, sham-controlled parallel group design. The aim of the study was to investigate the effect of iTBS on acute anxiety in spider phobia and the psychophysiological changes that go along with it. The following hypotheses were tested. (1) Watching a virtual spider scene provokes anxiety and disgust as well as the activation of the sympathetic nervous system as indicated by an increase of HR and SCL and, regarding HRV, an increase of the LF component accompanied by a decrease of the HF component in participants with spider phobia. (2) These emotional as well as psychophysiological reactions are less pronounced in healthy control participants. (3) ITBS attenuates the increase of anxiety, disgust, HR, SCL as well as the increase of the LF component and the respective decrease of the HF component in participants with spider phobia stimulated actively, but not in the sham group. (4) Participants with spider phobia display a stronger feeling of presence during virtual reality compared to healthy control participants.

3.1.3 Material and methods

3.1.3.1 Participants

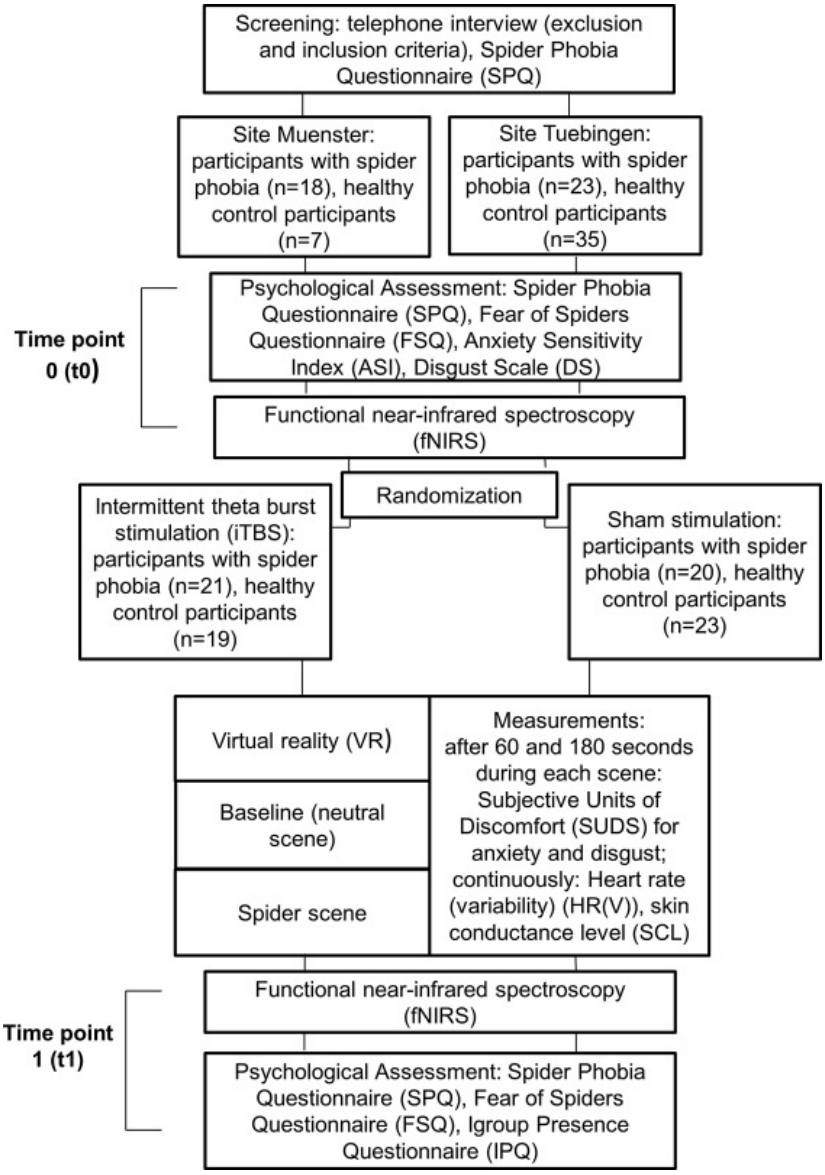
Participants with spider phobia and healthy controls were recruited via local advertisements. They had to be between 18 and 65 years of age. Participants with spider phobia had to fulfill DSM-IV-TR (American Psychiatric Association, 1995) diagnostic criteria of specific phobia for spiders (but did not necessarily need to be extremely restricted in their daily routine by their fear). Healthy participants were required to have no fear of spiders at all. Participants further filled in the German version of the spider phobia questionnaire (SPQ; Klorman, Weerts, Hastings, Melamed, & Lang, 1974; Watts & Sharrock, 1984) for screening, and were only included if they obtained 16 or more (participants with phobia), or 7 or less (healthy participants), of 31 possible points. If participants failed to fill in the SPQ during screening, the SPQ filled in at the beginning of the study ($t0$) was used for the decision instead. Exclusion criteria for all participants were pregnancy, severe

somatic disorders and current or previous psychiatric disorders other than specific phobia, intake of psychiatric or psychotropic medication and TMS contraindications (e.g., ferromagnetic implants). Diagnosis and comorbidity were assessed with the relevant section of the SCID interview (specific phobia)(First et al., 1995) and the M.I.N.I. (Sheehan et al., 1998) by a licensed psychologist or a medical doctor experienced in psychiatry. The study was approved by the local ethics committees of the Universities of Muenster and Tuebingen. Procedures were in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from all participants after detailed study description.

3.1.3.2 Design

A single-blind, randomized, sham-controlled parallel-group trial was conducted to test the clinical and neurobiological effects of iTBS in 41 subjects with spider phobia and 42 healthy participants during a virtual reality challenge. Participants in both groups were randomized either to active iTBS or to sham stimulation, resulting in four groups, the phobic actively stimulated group, the phobic sham-stimulated group, the healthy control actively stimulated group and the healthy control sham-stimulated group (see Figure 1).

Figure 1



Study design. Abbreviations: *t*0 and *t*1: measurement time points

Subjects visited the study center once. Before this visit, a telephone screening was conducted. In addition to the procedures reported here, in the beginning and in the end of the study a functional near-infrared spectroscopy (fNIRS) measurement was conducted during performance of an emotional Stroop task, the results of which are reported elsewhere.

3.1.3.3 Sample size calculation

Sample size was calculated with G*Power, provided freely by the University of Duesseldorf (www.gpower.hhu.de). In an earlier study investigating an acute intervention for participants with spider phobia exposed to the same VR scenario an effect size of 2.6 (Cohen's *d*) was observed (Diemer et al., 2013). Acting on the assumption of a somewhat lower *d* of 1.5, a sample size of at least 13 per group would have been necessary. We decided to have at least 19 in each group.

3.1.3.4 Self-report measures

Participants filled in the fear of spiders questionnaire (FSQ; Rinck et al., 2002; Szymanski & O'Donohue, 1995) and the spider phobia questionnaire (SPQ).

The questionnaires were administered at baseline and immediately after the second fNIRS measurement. At the latter assessment, subjects were instructed to rate symptoms retrospectively for the most aversive moment during the VR challenge. As for trait measures, we employed the anxiety sensitivity index (ASI; Reiss, Peterson, Gursky, & McNally, 1986), and the questionnaire for the assessment of disgust sensitivity (disgust scale: DS; Haidt, McCauley, & Rozin, 1994; Schienle, Walter, Stark, & Vaitl, 2002) at baseline only.

During VR, subjective units of discomfort scale (SUDS) for anxiety and disgust on a scale from 0 to 100, with 100 indicating maximum fear or maximum disgust, had to be reported orally 60 s and 180 s after the beginning of the respective scene (baseline scene and spider scene).

At the end, participants additionally filled in the Igroup Presence Questionnaire (IPQ; Schubert, Friedmann, & Regenbrecht, 2001). It consists of three subscales: namely *Spatial Presence*, the perception of the virtual space as a real space, *Involvement*, the level of attention towards the virtual reality, and *Experienced Realism*, the judgment, if virtual reality resembles the real world.

3.1.3.5 Electrophysiological measures

Electrodes for heart rate (HR) measurement (Red Dot®, 3 M) were positioned on the upper part of the body. Two electrodes (sintered Ag/AgCl electrodes) for skin conductance (electrodermal activity, EDA) measurement were placed on the thenar and hypothenar of the non-dominant hand. During VR, physiological measures (HR and EDA) were continuously monitored, using V-Amp 16 (BrainProducts, Gilching, Germany), and BrainVision Recorder Software (V-Amp Edition 1.10, Brain Products GmbH, Gilching, Germany).

3.1.3.6 iTBS

A single session of intermittent theta burst stimulation (iTBS) (described in greater detail by Huang et al., 2005) was conducted directly before the VR challenge. According to this protocol, a total of 600 pulses were applied in intermittent biphasic bursts at a frequency of 15 pulses per second via 2 s trains, starting every 10 s, resulting in a stimulation period of about three minutes. Stimulation site was F3 (left DLPFC) according to the international 10–20 system for electrode placement (Herwig et al., 2003). During verum stimulation, the coil was positioned tangentially to the scalp forming a 45° angle to the mid-sagittal line of the head with the handle pointing into a posterior direction. For sham stimulation, it was rotated by 90°, so the stimulation face was no longer in contact with the scalp. Although small effects on brain activity cannot be excluded by this method, it induces such effects to a lesser extent than other comparable methods and is therefore used routinely for sham stimulation (Lisanby, Gutman, Luber, Schroeder, & Sackeim, 2001). To take into account individual differences in cortical excitability, the participants' resting motor threshold was determined and the stimulation was conducted at 80% of that threshold.

3.1.3.7 Virtual reality challenge

The VR environment (created with Source Engine, Valve Corporation, Bellevue, Washington, USA) was presented monoscopically using a Z800 3D Visor head-mounted display (800 × 600 pixels, eMagin, Bellevue, Washington, USA), thereby generating the impression of a 3D environment. Head movements were assessed using the Patriot electromagnetic tracking device (Polhemus Corporation, Colchester, Vermont, USA). The simulation was controlled by the CyberSession software built at the Psychological Department of the University of Wuerzburg (www.cybersession.info). The VR challenge consisted of two scenes, each of which lasted for 180 s. A laboratory room was used as a neutral practice scene to allow familiarization with the virtual environment. Afterwards, this scene was shown again for baseline measurements of HR and SCL. In the following spider scene, participants saw three giant spiders crawling around the same laboratory room (Diemer et al., 2013).

3.1.3.8. Preprocessing of electrophysiological data

HR and SCL were preprocessed with BrainVision Analyzer Software 2.0 (Brain Products GmbH, Gilching, Germany). HR and SCL were analyzed after excluding speaking time for subjective ratings of anxiety and disgust. As a result, we analyzed two segments out of each scene (baseline scene and spider scene): the first 50 s (0–50 s, beginning of the scene) and the interval between 90 and 150 s (end of the scene). Mean HR (beats per minute) and mean skin conductance levels (per minute; SCL; µS) during the scenes were calculated for these segments. For analysis of mean HR low pass filter (high cut off frequency at 30 Hz) and high pass filter (low cut off frequency at 1.5915 Hz) were used. No filters were used for the analysis of mean SCL (Diemer et al., 2013). Data were screened for artefacts by visual examination which led to exclusion of the data from all participants recruited at the site of Muenster for further analysis of SCL. For HR as well as SCL, a few participants had to be excluded due to recording errors or bad data quality (see chapter "missing values"). For HRV, the first two and a half minutes of baseline and the first two and a

half minutes of the spider scene were analyzed without excluding speaking time, since segments would have been too short for a meaningful analysis of HRV otherwise. For calculation of parameters of HRV, Kubios HRV analysis software (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014) was used. The HRV parameters LF (mediated by the sympathetic and the parasympathetic nervous system) and HF (mediated mainly by the parasympathetic nervous system) were calculated by the software according to the frequency-domain method Welch's periodogram, whereby a spectrum estimate is calculated for the ECG interbeat intervals (described in greater detail by Tarvainen et al., 2014). Normalized units of LF and HF were defined according to Burr (2007) as the ratio of each component and total power of the individual participant (HFnu = HF/(HF + LF); LFnu = LF/(HF + LF)). Normalized units for LF and HF were used since normalized units are adjusted for the often large interindividual differences (see cf. Malik et al., 1996).

3.1.3.9 Statistical analyses

Statistical analyses were calculated with IBM SPSS Statistics 22. Participants were excluded from analysis of a questionnaire if they had left out more than two items. If participants had omitted one or two items, these were replaced by the mean of the respective (sub)scale. To sample characteristics, age and years of education, questionnaire data for *t*0 and *t*1, scores of ASI, DS and IPQ were compared directly via analysis of variance (ANOVA) with study group (participants with spider phobia vs. healthy controls) and treatment group (active iTBS vs. sham treatment) as between-subjects factors. $X2$-tests (gender, handedness and first language) were conducted in order to ensure that there was no significant difference between participants with spider phobia and healthy controls or the participants randomized to either sham or verum iTBS.

The effectiveness of blinding regarding treatment condition was evaluated using binomial tests (test proportion: 0.5) for the subjectively perceived condition, separately in each group of participants.

Mixed-design analysis of variance (ANOVA) of the scores of SPQ and FSQ was conducted with study group and treatment group as between-subjects factors and time (*t*0 and *t*1) as within-subjects factor. To avoid α-error accumulation due to multiple testing, the significance level of $\alpha = 0.05$ was adjusted using a Bonferroni correction procedure for this analysis resulting in a level of $\alpha = 0.025$ (0.05/2).

Mixed-design ANOVA was also conducted for SUDS, SCL and HR with study group and treatment group as between-subjects factors and scenario (baseline and spider scene) as well as duration (beginning of the scene and end of the scene) as within-subjects factors. For the HRV sub-measures LF and HF, a mixed-design ANOVA was conducted in almost the same manner, but without the factor duration. If significant three-way-interactions occurred, post-hoc-ANOVAs were conducted for groups separately. Two-tailed *t*-tests were used to further explore main effects and two-way-interactions.

Since not all scores were distributed normally according to Kolmogorov–Smirnov tests, Mann–Whitney-*U*-test or Wilcoxon-test as non-parametric tests were conducted post-hoc for all analyses which included scores not distributed normally. If results of non-parametric tests deviated from results of ANOVAs, these deviations are reported. As an additional post-hoc test for sub-measures of HRV, differences between spider scene and baseline were calculated for LF and HF and a univariate ANOVA with treatment group as between-subjects factor was conducted for these differences.

3.1.3.10 Missing values

SPQ and FSQ at *t*0 were available for 81 participants. SPQ at *t*1 was available for 81, FSQ for 82 participants. There were a relatively high amount of missing values (12) for the question of whether participants felt they were in the sham or verum iTBS condition. It is not clear why participants may have overlooked this question. SUDS were available for 80 participants. Heart rate data were available for the beginning of the spider scene for 80 participants and for 79 participants for all other time points during the scenes. LF and HF were available for 78 participants at baseline and for

79 participants during the spider scene. For analysis of mean skin conductance level (SCL) participants from the site of Muenster had to be excluded due to a technical problem. Measurements were available for 55 participants whereof 23 were participants with spider phobia (verum 12, sham 11) and 32 were healthy controls (verum 14, sham 18). The IPQ was available for 80 participants.

3.1.4 Results

3.1.4.1 Sample characteristics

There were no differences in sociodemographic data (age, handedness, gender, first language and years of education) between the four groups except for significantly more left-handed participants in the actively stimulated iTBS compared to the sham group (see Table 1). As expected, SPQ and FSQ at baseline revealed significantly higher scores in participants with spider phobia than in healthy controls (see Table 2).

Table 1

Study group	Total (n=83)	Partici-pants with spider phobia (n=41)	Healthy controls (n=42)	iTBS (n=40)	Sham (n=43)	Healthy controls vs. patients	iTBS vs. Sham
Age Mean (SD)	26.46 (8.47)	27.51 (9.45)	25.43 (7.37)	25.85 (7.65)	27.02 (9.23)	F(1,79)=1.25, p=0.267	F(1,79)=0.50, p=0.481
Gender	9 male, 74 female	4 male, 37 female	5 male, 37 female	4 male, 36 female	35 male, 38 female	x_1^2=0.10, p=0.753	x_1^2= 0.057, p=0.812
Handed-ness	12 left, 71 right	6 left, 35 right	6 left, 36 right	9 left, 31 right	3 left, 40 right	x_1^2= 0.002, p=0.964	x_1^2 = 4.04, p= 0.044*
First Lan-guage	74 German, 5 bilingual, 4 other	35 German, 4 bilingual, 2 other	39 German, 1 bilingual, 2 other	34 German, 3 bilingual, 3 other	40 German, 2 bilingual, 1 other	x_2^2=2.00, p=0.367	x_2^2=1.58, p=0.454
Years of edu-cation Mean (SD)[a]	11.32 (3.68)	11.00 (2.90)	11.66 (3.34)	11.30 (3.91)	11.34 (3.51)	F(1,78)=0.57, p=0.451	F(1,78)=0.01, p=0.948

Table 1. Sociodemographic Data. a = available for 82 participants only; *significant on the level p<0.05; SD = standard deviation; z = two-sided significance according to Fisher's exact test.

Table 2

Study group	Total (n=83)	Partici-pants with spider phobia (n=41)	Healthy controls (n=42)	iTBS (n=40)	Sham (n=43)	Healthy controls vs. patients	iTBS vs. Sham
SPQ[a] (SD)	12.13 (10.08)	21.81 (3.38)	2.68 (2.62)	12.31 (10.39)	11.96 (9.91)	F(1,77)= 804.13, p<0.001***	F(1,77)= 0.271, p=0.604
FSQ[a] (SD)	38.18 (37.46)	74.42 (13.95)	2.83 (4.82)	39.54 (38.49)	36.92 (36.91)	F(1,77)= 940.39, p<0.001***	F(1,77)< 0.001, p=0.997
ASI (SD)	14.98 (7.95)	17.68 (8.56)	12.33 (6.35)	14.70 (7.62)	15.23 (8.32)	F(1,79)= 10.35, p=0.002**	F(1,79)=0.265, p=0.608
DS (SD)	77.32 (22.34)	82.19 (23.00)	72.67 (20.87)	74.46 (23.36)	79.98 (21.29)	F(1,79)= 4.19, p=0.044*	F(1,79)= 1.61, p=0.208

Table 2. a = available for 81 participants only; *significant on the level p<0.05; **significant on the level p<0.01; ***significant on the level p<0.001; SD = standard deviation

Participants with spider phobia were significantly more sensitive than healthy controls to anxiety (according to ASI) as well as disgust (according to DS, see Table 2).

3.1.4.2 Effectiveness of blinding

Participants were asked to guess whether they had been treated with active or sham iTBS. The guesses did not differ significantly from chance for any of the four groups (results of binomial tests: participants with spider phobia active iTBS $p = 1.00$; participants with spider phobia sham $p = 1.00$; healthy control participants active iTBS $p = 0.18$; healthy control participants sham $p = 0.52$). In the total group 37 answers were available for participants who had been sham-stimulated of which 21 guessed this correctly and 16 assumed that they had received active iTBS. Of the 34 actively stimulated participants that answered the question, 14 believed to have been sham-stimulated, while 20 stated to have received active iTBS.

3.1.4.3 Changes of spider phobia questionnaires over time

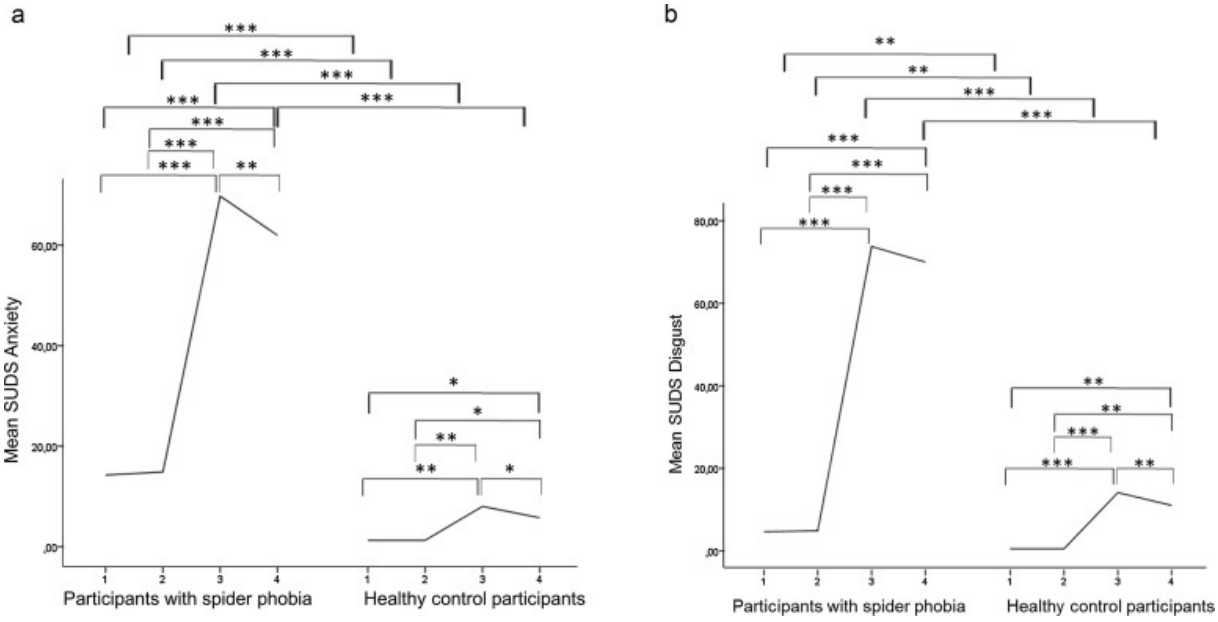
Level of significance was adjusted from $p = 0.05$ to $p = 0.025$ (Bonferroni correction), because two spider phobia questionnaires were included in the analysis. Mean values of the SPQ did not change significantly over time ($F(1,77) = 3.25$, $p = 0.075$). For the FSQ, there were a significant main effect of time ($F(1,77) = 8.55$, $p = 0.005$) and an interaction of time and study group ($F(1,77) = 7.46$, $p = 0.008$) due to a decrease of the score from $t0$ to $t1$ in the spider phobic group, but not in the control group (phobic group $t(39) = 3.01$, $p < 0.005$; control group $t(40) = 0.29$, $p = 0.774$). There was a main effect of study group for both questionnaires (SPQ: $F(1,77) = 922.32$, $p < 0.001$; FSQ: $F(1,77) = 762.07$, $p < 0.001$) due to higher mean values in the spider phobic group compared to the control group. No differences occurred between the sham and the active iTBS group.

3.1.4.4 SUDS

Fig. 2(a and b) gives an overview over changes of anxiety and disgust over time. Significantly more anxiety and disgust were perceived by participants during spider scene than during baseline (main effect of scenario: anxiety $F(1,76) = 133.53$, $p < 0.001$; disgust $F(1,76) = 272.22$, $p < 0.001$). Mean anxiety and disgust were significantly stronger in the beginning compared to the end of spider scene (main effect of duration: anxiety $F(1,76) = 7.83$, $p = 0.007$; disgust $F(1,76) = 5.20$, $p = 0.025$; interaction of scenario and duration: anxiety $F(1,76) = 14.15$, $p < 0.001$; disgust $F(1,76) = 5.97$, $p = 0.017$). Overall, significantly more anxiety and disgust were reported by participants with spider phobia than by healthy controls (main effect of study group: anxiety $F(1,76) = 133.05$, $p < 0.001$; disgust $F(1,76) = 163.34$, $p < 0.001$). In participants with spider phobia anxiety and disgust increased more strongly from baseline to spider scene than in healthy control participants (significant interaction of scenario and study group: anxiety $F(1,76) = 85.48$, $p < 0.001$; disgust $F(1,76) = 130.28$, $p < 0.001$). Anxiety decreased significantly more strongly from the beginning to the end of spider scene

in the phobic group compared to the healthy control group (three-way-interaction of duration × scenario × study group $F(1,76) = 4.73$, $p = 0.033$). There was no significant main or interaction effect of iTBS on SUDS.

Figure 2



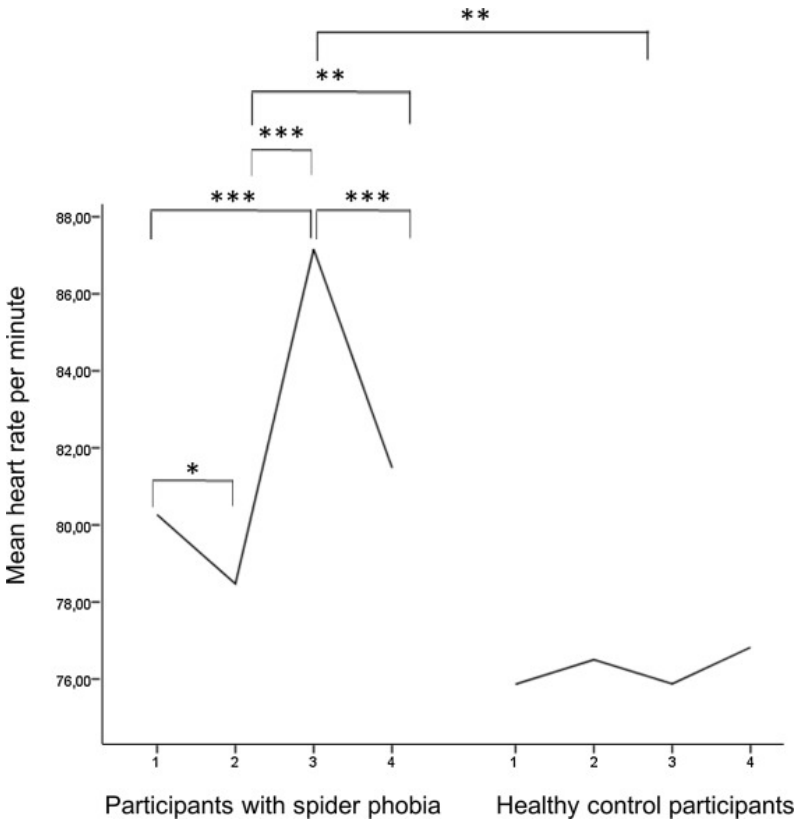
(a) Anxiety during virtual reality. 1 = Beginning of baseline; 2 = end of baseline; 3 = beginning of spider scene; 4 = end of spider scene; * significant on the level $p < 0.05$; ** significant on the level $p < 0.01$; *** significant on the level $p < 0.001$. (b) Disgust during virtual reality. 1 = Beginning of baseline; 2 = end of baseline; 3 = beginning of spider scene; 4 = end of spider scene; * significant on the level $p < 0.05$; ** significant on the level $p < 0.01$; *** significant on the level $p < 0.001$.

3.1.4.5 Heart rate

Fig. 3 gives an overview about changes of HR over time. The phobic group only displayed a significantly higher heart rate during spider scene compared to baseline (main effect of scenario: $F(1,74) = 24.71$, $p < 0.001$; significant interaction of scenario and study group: $F(1,74) = 20.46$, $p < 0.001$). A significantly higher heart rate at the beginning of the spider scene and baseline compared to the end of the respective scene was also observed in participants with spider phobia only (main effect of duration: $F(1,74) = 10.41$, $p = 0.002$; significant interaction of duration and study group: $F(1,74) = 22.08$, $p < 0.001$). This decrease from the beginning to the

end of the scene was significantly more pronounced in the spider scene (three-way interaction scenario × duration × study group: $F(1,74) = 4.91$, $p = 0.030$). There was no significant main or interaction effect of iTBS on HR.

Figure 3



Heart rate during virtual reality. 1 = Beginning of baseline; 2 = end of baseline; 3 = beginning of spider scene; 4 = end of spider scene; * significant on the level $p < 0.05$; ** significant on the level $p < 0.01$; *** significant on the level $p < 0.001$

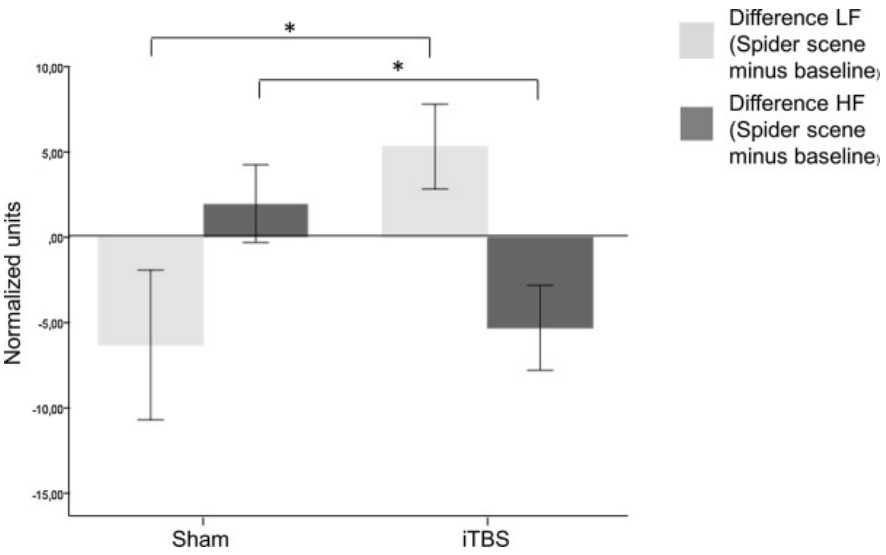
3.1.4.6. Heart rate variability

Mixed-design ANOVAs revealed a significant interaction of scenario and treatment group (verum vs. sham) for HF and LF, displayed as normalized units (HF $F(1,73) = 4.17$, $p = 0.045$; LF $F(1,73) = 4.96$, $p = 0.029$). This was due to a significant increase of LF and a decrease of HF, thus an increase of sympathetic influence, in the active iTBS group only during the spider scene. Since data were not

distributed normally according to Kolmogorov–Smirnov tests, Wilcoxon tests were conducted which were only marginally significant (LF $z = 1.88$, $p = 0.06$; HF $z = 1.88$, $p = 0.06$).

Differences between spider scene and baseline were calculated. ANOVA revealed a significant main effect of treatment group (iTBS vs. sham) for both differences (LFdiff $F(1,73) = 5.23$, $p = 0.025$; HFdiff $F(1,73) = 4.67$, $p = 0.034$; see Fig. 4). LFdiff and HFdiff were distributed normally according to Kolmogorov–Smirnov in the iTBS group. No main or interaction effect occurred according to study group.

Figure 4



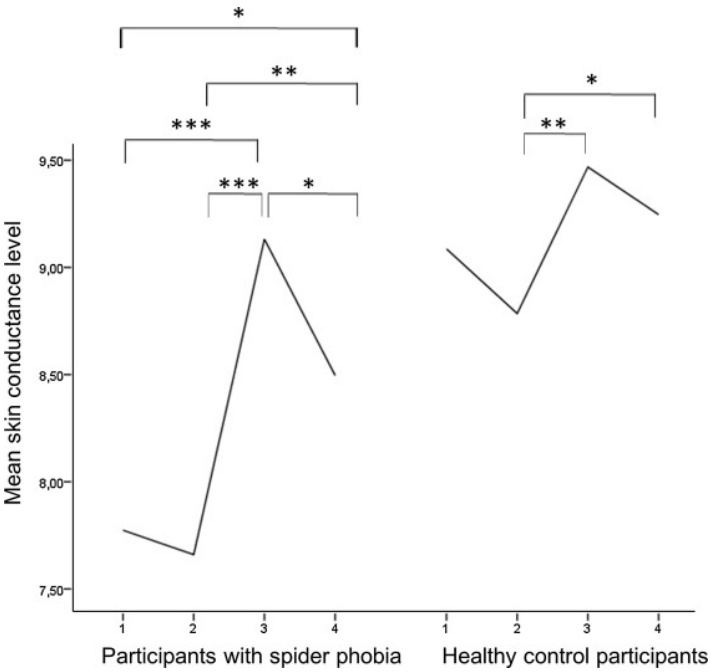
Parameters of heart rate variability LF and HF during virtual reality. * Significant on the level $p < 0.05$. Error bars represent one standard error of the mean.

3.1.4.7 Skin conductance

Fig. 5 gives an overview about changes of SCL over time. Mean SCL was significantly higher during the spider scene than during baseline (main effect of scenario: $F(1,51) = 27.93$, $p < 0.001$). This increase was more pronounced in the phobic group compared to the control group (interaction of scenario and study group: $F(1,51) = 5.17$, $p = 0.027$). There was a higher SCL at the beginning compared to

the end of both scenes (main effect of duration: F(1,51) = 15.92, p < 0.001). No main or interaction effects occurred according to iTBS.

Figure 5



Skin conductance level during virtual reality. 1 = Beginning of baseline; 2 = end of baseline; 3 = beginning of spider scene; 4 = end of spider scene; * significant on the level $p < 0.05$; ** significant on the level $p < 0.01$; *** significant on the level $p < 0.001$.

3.1.4.8. Presence

There was a main effect of study group on presence. The experience of presence, measured by the IPQ, was significantly stronger in the phobic group (F(1,76) = 9.25, p = 0.003). This was due to the subscales Involvement (F(1,76) = 8.29, p = 0.005) and Experienced Realism (F(1,76) = 10.18, p = 0.002), while the subscale Spatial Presence showed no significant difference between groups (F(1,76) = 1.34, p = 0.250).

3.1.5 Discussion

In accordance with our first hypothesis, the virtual reality (VR) spider challenge led to subjective anxiety and disgust as well as elevated heart rate (HR) and skin conductance level (SCL) in participants with spider phobia. However, there was no general change of heart rate variability (HRV) in terms of the LF and HF component in the phobic group during the challenge. Surprisingly, regarding fearfulness towards spiders, as measured by the fear of spiders questionnaire (FSQ), the phobic group scored significantly lower judging their feelings during virtual reality compared to the beginning of the study. In line with our second hypothesis, anxiety, disgust and elevated SCL were provoked to a lesser extent in healthy participants. Although not explicitly intended, anxiety, disgust, HR and SCL decreased from the beginning to the end of the spider scene in the whole group of participants. Contrary to our third hypothesis, we found no general effect of active intermittent theta burst stimulation (iTBS) on subjective anxiety, HR, and SCL. Interestingly; however, active iTBS significantly increased sympathetic activity (increase of LF and decrease of HF) during the spider scene. In accordance with our forth hypothesis, the experience of presence during VR was significantly stronger in participants with spider phobia than in healthy controls according to the subscales *Involvement* and *Experienced Realism* of the IPQ.

In more detail, increases of anxiety and disgust were observed as expected and were hence in line with earlier studies on VR challenges in patients with specific phobia (e.g., Diemer et al., 2013; Muhlberger, Petrusek, Herrmann, & Pauli, 2005). The same holds true for increases of HR and SCL. In this study, HR differentiated better than SCL between participants with spider phobia and healthy controls, a result that has not been shown in other studies (e.g., Diemer et al., 2013) and might be due to the smaller number of participants that could be included in the analysis of SCL. However, the property of HR to differentiate accurately between participants with phobia and healthy controls during a VR challenge has been demonstrated before (Mühlberger, Bülthoff, Wiedemann, & Pauli, 2007). The decrease of the FSQ score was surprising to us, since participants were asked to rate their feelings towards spiders at the time during VR they felt the worst. A possible explanation might be that participants confused their feelings during and after the session. The latter time

might have been characterized by relief. This view is supported by the fact that anxiety and disgust strongly increased during the spider scene compared to baseline, while only the FSQ filled in with some latency after the challenge displayed a decrease. In any case, informative value of this decrease is limited, since the questionnaire was developed for the assessment of fear of spiders in general and not for short-term changes.

Healthy participants reporting no fear of spiders in general and no or minimal anxiety or disgust during the spider scene nevertheless display psychophysiological arousal. While this remains an unanswered phenomenon, one possible explanation is that the arousal is caused by interest in the new situation, increased attention or elation, and not by fear.

There was a decrease of anxiety and disgust as well as HR and SCL from the beginning to the end of the spider scene that can be interpreted as habituation. This is noteworthy, since participants were only exposed once, the duration of the session was relatively short (three minutes) and they had received no specific instruction before the challenge. However, it cannot be excluded that this decrease was caused by the knowledge of the participants that the end of the session was near, rather than by a real habituation effect.

The increase of LF and decrease of HF displayed by the actively stimulated iTBS group during the spider scene is contrary to our expectation and to the best of our knowledge the first evidence for a short term effect of iTBS on HRV changes provoked by acute anxiety and disgust. If iTBS over the left PFC had the potential to attenuate arousal or anxiety, we would have expected decreased sympathetic and increased parasympathetic activity. This view is supported by results of Udupa et al. who reported just the opposite of our short term effect, namely a decrease of LF and an increase of HF succeeding long term repeated high-frequency rTMS over the left DLPFC (Udupa et al., 2007; Udupa et al., 2011). However, participants in this study were not exposed to a stressful situation or a stimulus that provoked anxiety and no theta burst stimulation was used. Effects of iTBS may depend on other factors influencing HRV at the same time and acute effects may differ from, or be even contrary to, long term effects. Though highly speculative, the iTBS induced increase of LF and decrease of HF could also be interpreted as increased attention towards, or

increased salience of, the presented stimuli. The latter was observed by Shahbabaie et al. (2014) after transcranial direct current stimulation over the left DLPFC in combination with exposure to a challenging situation, though the context of their study was a different one.

Still, since reports on short-term effects of rTMS are up to now rather ambiguous (Vernieri et al., 2014; Yoshida et al., 2001), our study adds another piece of evidence to the complex picture of neurostimulation. Based on this, further research is necessary to better identify possible mechanisms by which TMS influences HRV and which parameters determine the direction of this influence.

The significantly stronger experience of presence in participants with spider phobia is in accordance with earlier studies correlating presence with strong emotions as well as arousal (Diemer et al., 2015). Our results regarding the subscales of the IPQ suggest that this connection is not related to the experience of the virtual room as a real space (*Spatial Presence*), but to other aspects of presence (*Involvement* and *Experienced Realism*). To our knowledge, this is the first study that used the IPQ subscales in specific phobia. The results are partially in accordance with Price, Mehta, Tone, & Anderson (2011) who found a correlation of peak anxiety with *Experienced Realism*, but not with *Involvement* and *Spatial Presence* in patients with social phobia exposed to a virtual reality scenario.

Finally, some limitations and considerations need to be mentioned. Firstly, iTBS was only applied once, which might not be enough to attenuate anxiety. Future research should therefore try to evaluate the effectiveness of repeated iTBS applications in anxiety disorders. Blinding was successful, which can be considered a strength of the study. Still, some participants complained about awkward sensations or even pain during active iTBS which might have influenced the perception of the subsequent VR, e.g., in a way that recipients of active iTBS felt more tensed or stressed and therefore also perceived VR to be more stressful. Baseline measurements of HR, HRV and SCL were recorded after iTBS only, which was due to the complexity of our design, but still limits the interpretation of iTBS effects on these parameters. Future studies therefore should focus on comparison of HR, HRV and SCL before and after iTBS. Questionnaires for the assessment of VR were not handed out immediately afterwards. Rather, participants attended the second functional near-infrared

spectroscopy first. It cannot be excluded that their evaluation of VR was changed by this interruption or that it became less precise due to the time delay.

3.1.6 Conclusion

Summarizing our results, VR is an effective method to provoke anxiety as well as disgust and psychophysiological arousal in participants with spider phobia. Compared to earlier VR studies showing these effects (cf. Freire, De Carvalho, Joffily, Zin, & Nardi, 2010; Mühlberger et al., 2007; Wiederhold, Jang, Kim, & Wiederhold, 2002), our study comprised a larger sample and investigated changes of anxiety, disgust, HR and SCL over time thereby detecting an early decrease of these parameters, interpreted as a habituation effect. We conclude that VR in participants with spider phobia is a good model of acute anxiety that allows a differentiated investigation of this phenomenon.

To our knowledge this is the first study probing iTBS in a model of acute anxiety. A single session of iTBS had no effect on the subjective and psychophysiological reactions provoked by VR. It cannot be decided whether more sessions would have been effective, or whether this TMS protocol is in general not appropriate to influence strong, acute anxiety. Future studies should also focus on other target regions and TMS protocols. Low frequency rTMS of the right DLPFC displayed anxiolytic properties in preclinical and clinical studies (Lefaucheur et al., 2014; Zwanzger et al., 2009). Different forms of TMS targeting the medial prefrontal cortex have shown promising results in posttraumatic stress disorder (Isserles et al., 2013), obsessive compulsive disorder (Modirrousta et al., 2015)and in modulating the processing of conditioned fear (Guhn et al., 2014).

ITBS led to a sympathetic reaction towards the spider scene according to HRV. Future studies should therefore further examine the influence of different TMS protocols and stimulation sites on HRV.

Regarding presence, this study confirmed the link between presence and arousal. It provides preliminary evidence that this link is based on involvement and experienced realism during VR and not so much on the experience of VR as a real space.

3.2 Manuscript 2: Functional co-activation within the PFC supports the maintenance of behavioural performance in fear-relevant situations – an iTBS modulated virtual reality challenge with spider phobic participants

The contents of this chapter are published in:

Deppermann, S.*, Notzon, S.*, Kroczek, A., Rosenbaum, D., Haeussinger, F. B., Diemer, J., Domschke K., Fallgatter A. J., Ehlis A.-C. & Zwanzger, P. (2016). Functional co-activation within the prefrontal cortex supports the maintenance of behavioural performance in fear-relevant situations before an iTBS modulated virtual reality challenge in participants with spider phobia. Behavioural brain research, 307, 208-217.

* Both authors contributed equally to this work

3.2.1 Abstract

A number of studies/meta-analyses reported moderate antidepressant effects of activating repetitive transcranial magnetic stimulation (rTMS) over the prefrontal cortex (PFC). Regarding the treatment of anxiety, study outcomes are inconsistent, probably because of the heterogenity of anxiety disorders/study designs. To specifically evaluate the impact of rTMS on emotion regulation in fear-relevant situations we applied a sham-controlled activating protocol (intermittent Theta Burst Stimulation/iTBS) over the left PFC (F3) succeeded by a virtual reality (VR) challenge in n = 41 participants with spider phobia and n = 42 controls. Prior to/after iTBS and following VR prefrontal activation was assessed by functional near-infrared spectroscopy during an emotional Stroop paradigm. Performance (reaction times/error rates) was evaluated. Stimuli were rated regarding valence/arousal at both measurements.

We found diminished activation in the left inferior frontal gyrus (IFG) of participants with spider phobia compared to controls, particularly elicited by emotionally-irrelevant words. Simultaneously, a functional connectivity analysis showed increased co-activation between the left IFG and the contra-lateral hemisphere. Behavioural performance was unimpaired. After iTBS/VR no significant differences in cortical activation between the phobic and control group remained. However, verum iTBS did not cause an additional augmentation. We interpreted our results in terms of a prefrontal network which gets activated by emotionally-relevant stimuli and supports the maintenance of adequate behavioural reactions. The missing add-on effects of iTBS might be due to a ceiling effect of VR, thereby supporting its potential during exposure therapy. Concurrently, it implies that the efficient application of iTBS in the context of emotion regulation still needs to be studied further.

3.2.2 Introduction

With a life time prevalence of up to six percent, spider phobia is the most common specific phobia of the animal type which mostly affects women (Fredrikson et al., 1996). As in other anxiety disorders, an inadequate top-down regulation of subcortical structures such as the modulation of the amygdala by the prefrontal cortex (PFC) is assumed to be a core feature (Carlsson et al., 2004; Dilger et al., 2003; Gorman et al., 2000; Johanson et al., 2006; Schienle, Schäfer, Hermann, Rohrmann, & Vaitl, 2007). While the amygdala is thought to be associated with vigilance and flight reaction (LeDoux, 2001; Öhman & Mineka, 2001) the PFC is involved in the regulation of emotion (Goldin et al., 2008; Thier, 2006). The dorsolateral prefrontal cortex (DLPFC) specifically, as a main neural correlate of executive function, plays an important role regarding goal-oriented inhibition of emotional responses (Buhle et al., 2014; Delgado, Nearing, LeDoux, & Phelps, 2008). While the inferior frontal gyrus (IFG) comprises a major part of Broca's area, known for its role in language processing (Friederici, 2011), it could also be linked to emotion regulation (Goldin et al., 2008), as well as response suppression and attentional control, especially but not exclusively in the non-dominant hemisphere (Hampshire et al., 2010; Swick et al., 2008).

Diverging prefrontal brain activation patterns have been observed in response to fear-inducing stimuli. While some studies found prefrontal hypoactivation (Carlsson et al., 2004; Johanson et al., 2006; Schienle et al., 2007) other findings showed enhanced activation (Paquette et al., 2003; Schienle et al., 2005; Straube et al., 2004). An explanation for these controversial outcomes might be the different cognitive processes involved in the specific tasks applied.

Within this framework, the "Valence hypothesis" is a neurobiological model which postulates hypo- as well as prefrontal hyperactivation depending on the context of the task. In accordance with this hypothesis, the left hemisphere plays a major role in the modulation of approach related emotions while the right hemisphere mainly modulates avoidance related emotions (Vennewald et al., 2013).

Bringing together the valence hypothesis and the model of inadequate top-down regulation found in anxiety disorders, we decided to apply an activating repetitive

transcranial magnetic stimulation (rTMS) protocol on the left hemisphere in order to enhance prefrontal activation and associated cognitive control during a situation which induces avoidance related emotions.

Intermittent theta burst stimulation iTBS (Huang et al., 2005), is a modern form of activating rTMS which is able to modulate cortical activation non-invasively by electro-magnetic induction. Compared to traditional rTMS, a longer-lasting effect is obtained with shorter stimulation. In this context, studies point to an enhancing effect of approximately an hour after a one-time application of an iTBS protocol (Grossheinrich et al., 2009).

So far, a number of studies and meta-analyses have found moderate antidepressant effects using repeated rTMS (O'Reardon et al., 2007). In the context of anxiety disorders, far fewer studies exist and results are inconsistent (Machado et al., 2012). This might be due to the complexity of some of these study designs where many active factors may have led to confounding effects. For example, in a previous study conducted by our workgroup (see section 2.1), panic disorder patients received repeated iTBS application while taking part in cognitive behavioural group therapy. To simplify the interpretation of study outcomes it might, however, be easier to look at an assessable number of specific factors separately.

Recently, virtual reality (VR) exposure hasbecome more established as an alternative to in-vivo exposition in the treatment of pathological fear. Its advantages include a better controllability of the therapeutic setting as well as a high compliance with patients (Powers & Emmelkamp, 2008). At the same time, studies have shown that VR is able to induce a significant increase in subjectively received fear as well as psychophysiological arousal (heart rate, skin conductance, Diemer et al., 2013; Diemer et al., 2014; Freire et al., 2010).

Within the framework of psychiatric research, functional near-infrared spectroscopy (fNIRS) is an imaging method with particularly good acceptance among participants due to its uncomplicated and fast application compared to other imaging methods, e.g. functional magnetic resonance imaging (fMRI) (Ehlis et al., 2014). As an optical imaging method, fNIRS exploits the fact that near-infrared light can penetrate scalp and skull. Because the chromophores oxyhaemoglobin (O2Hb) and

deoxyhaemoglobin (HHb) have distinct absorption spectra in the near-infrared range, it is possible to deduce regional oxygenation patterns by measuring the relative amount of reflected light (Ferrari & Quaresima, 2012), with a spatial resolution of approximately 3 cm and a depth penetration of about 2.5 cm (Haeussinger et al., 2011).

Altogether, this study aimed at examining the impact of one-time iTBS application over the PFC prior to a fearful situation. To do so, a VR environment was used to confront participants suffering from spider phobia with virtual spiders after receiving sham-controlled iTBS. Before and after the iTBS/VR combination, prefrontal activation was assessed by fNIRS while the participants completed an emotional Stroop paradigm.

The advantage of an emotional Stroop paradigm is that participants are not asked to willingly influence their emotions (potentially triggering more individual strategies and therefore more diverging results) but still need to ignore phobic or fearful content of the presented stimuli if they want to complete the task in an adequate manner.

In this regard, a number of authors (Dresler et al., 2012; Tupak, Reif, et al., 2013) reported increased activation in the IFG and other prefrontal areas elicited by anxiety provoking words during an emotional-word Stroop paradigm in a sample of patients with panic disorder. On a behavioural level, the difficulty of focusing on the mere task without getting distracted by anxiety-provoking stimuli is further supported by the fact that diverging reaction times (RTs) in emotional Stroop tasks have been shown (Dresler et al., 2012; Kindt & Brosschot, 1997; Mathews & Klug, 1993).

Specifically, in this paper we report the results concerning the following hypotheses:

a.) Phobic participants require more cognitive control when trying to respond to phobia-related stimuli in an adequate manner and therefore display increased DLPFC/IFG activation patterns.

b.) At the same time, we still expect decreased behavioural performance (RTs, error rates) to fear-related stimuli compared to healthy controls reflecting the difficulties in implicit emotion regulation during the confrontation with phobic words.

c.) ITBS followed by a VR challenge further promotes prefrontal activation elicited by emotional stimuli in participants with spider phobia and is associated with an improved behavioural outcome as well as a temporary adjustment of subjectively perceived valence and arousal ratings of the presented words.

d.) In addition to a standard fNIRS analysis of cortical activation patterns, we also investigated the functional connectivity within the PFC in order to get a better understanding of the interplay of the different sub-regions during emotional control processes.

3.2.3 Materials and methods

3.2.3.1 Subjects

Forty-one participants with spider phobia and 42 healthy controls were included in the study after written informed consent was obtained. All phobic participants fulfilled the DSM-IV criteria for specific spider phobia except that their day-to-day functioning did not have to be overly impeded by their fear of spiders. They further had to score on at least 16 of 31 possible items on the German version of the spider phobia questionnaire Watts and Sharrock, (Watts & Sharrock, 1984) while control subjects had to remain under 7 points at screening.

Subjects were excluded if they suffered from any psychiatric disorder other than specific phobia, organic brain disorder, another severe somatic illness or hypertension at the time of screening. Furthermore, pregnancy and lactation had to be ruled out and all subjects had to be between 18 and 65 years of age.

Phobic and control participants did not differ significantly according to age, gender, handedness, education and first language (see Table 1). The study was approved by the Ethics Committees of the Universities of Muenster and Tuebingen and all procedures were in accordance with the latest version of the Declaration of Helsinki.

Table 1

Study group	Total (n=83)	Partici-pants with spider phobia (n=41)	Healthy controls (n=42)	iTBS (n=40)	Sham (n=43)	Healthy controls vs. patients	iTBS vs. Sham
Age Mean (SD)	26.46 (8.47)	27.51 (9.45)	25.43 (7.37)	25.85 (7.65)	27.02 (9.23)	F(1,79)=1.25, p=0.267	F(1,79)=0.50, p=0.481
Gender	9 male, 74 female	4 male, 37 female	5 male, 37 female	4 male, 36 female	35 male, 38 female	x_1^2=0.10, p=0.753	x_1^2= 0.057, p=0.812
Handed-ness	12 left, 71 right	6 left, 35 right	6 left, 36 right	9 left, 31 right	3 left, 40 right	x_1^2= 0.002, p=0.964	x_1^2 = 4.04, p= 0.044*
First Lan-guage	74 German, 5 bilingual, 4 other	35 German, 4 bilingual, 2 other	39 German, 1 bilingual, 2 other	34 German, 3 bilingual, 3 other	40 German, 2 bilingual, 1 other	x_2^2=2.00, p=0.367	x_2^2=1.58, p=0.454
Years of edu-cation Mean (SD)[a]	11.32 (3.68)	11.00 (2.90)	11.66 (3.34)	11.30 (3.91)	11.34 (3.51)	F(1,78)=0.57, p=0.451	F(1,78)=0.01, p=0.948

Table 1. Sociodemographic Data. a = available for 82 participants only; *significant at the level p<0.05; SD = standard deviation

3.2.3.2 Design

The bicentric study (Muenster and Tuebingen) was conducted in a single-blind randomized sham-controlled group design. Phobic participants and controls participated in an fNIRS measurement before receiving either verum or sham iTBS (t1) which was followed by a VR challenge. During the VR objective (heart rate, skin conductance) as well as subjective (ratings of anxiety and disgust) parameters of the individual's fear reaction were collected (results are reported elsewhere) Subsequently, the fNIRS measurement was repeated (t2). A flow chart of the study is depicted in section 3.1.3.2.

3.2.3.3 Stimuli

During the fNIRS measurements all participants completed an emotional-word Stroop paradigm. In this regard, participants had to indicate via button press whether the presented words were written in red, green or blue, independent of their emotional content (10 emotionally positive, 10 emotionally negative, 10 neutral and 10 phobia related words). Beforehand, all words were matched with respect to the number of letters and syllables as well as corpus-based word frequency. Additionally, a pilot study was conducted where the words were rated on a 5-point scale to ensure that valence and arousal were induced as intended according to the word category and did not differ between groups except for the phobic words. After completing the fNIRS measurements, all participants also rated the presented words accordingly. During the experiment the stimuli were presented in a randomized order on a black LCD screen whereby each stimulus was presented for 1500 ms and was preceded by a fixation cross, which lasted 500 ms. The inter-trial intervals were randomly jittered between 4000 and 8000 ms.

3.2.3.4 fNIRS

The ETG-4000 continuous Optical Topography System (Hitachi Medical Co., Japan) was used for all fNIRS measurements to record relative changes in O2Hb and HHb concentration. To do so, we oriented the probe set (3 × 11 optodes array consisting of 16 photo detectors and 17 light emitters resulting in 52 channels) with its central optode of the lowest row on FPz reaching out towards T3 and T4 according to the international 10–20 EEG system for electrode placement (Jasper, 1958a). The ETG-4000 uses near-infrared light of two wavelengths which are modulated at a distinctive frequency for each channel (695 ± 20 nm and 830 ± 20 nm). The sampling rate was 10 Hz. After the photo detectors received the scattered light it was transferred to a set of lock-in amplifiers in order to separate it with respect to its modulation frequency and to analyse and transform it according to its wavelength. The calculated time course of O2Hb and HHb concentration changes was exported in

mmol × mm which implies that changes in O2Hb and HHb depend on the unknown path length of the near-infrared light in the tissue.

3.2.3.5 iTBS

Directly after the first fNIRS measurement (t0), a single (verum or sham) iTBS session (for further details on the stimulation protocol please refer to Ref. (Huang et al., 2005) was applied over the left PFC at F3 of the international 10–20 EEG system according to Herwig et al. (2003). In line with the findings on prefrontal hypo activation mentioned in the introduction as well as the "valence hypothesis" (e.g. [56] which assumes a hemispheric lateralisation whereby approach related emotions are modulated on the left and withdrawal related emotions on the right hemisphere, we chose the left PFC as our stimulation site. For the application, a figure-of-eight coil (MCF-B65, 2 × 75 mm diameter) was positioned tangentially to the scalp forming a 45° angle to the mid-sagittal line of the head with the handle pointing in a posterior direction. The sham stimulation was achieved by turning the coil at a 90° angle away from the scalp. The used stimulator was a MagOption/MagPro X100 stimulator (MagVenture, Denmark) with the stimulation intensity set to 80% of the individual's motor threshold. The iTBS was conducted directly prior to a spider-related VR challenge.

3.2.3.6 VR challenge

For the presentation of the virtual spider environment a head mounted display (HMD, Z800 3D Visor, 800 × 600 pixels, eMagin, Bellevue, Washington, USA) was used.

The head position was registered using the 6DOF (Polhemus Corporation, Colchester, Vermont, USA) electro-magnetic tracking system by means of a head set. Experimental control was achieved via the CyberSession software programmed at the Psychological Department of the University of Wuerzburg (www.cybersession.info) which has previously successfully been used to induce anxiety in VR challenges

(Diemer et al., 2013). During the course of the VR confrontation, all participants first entered a neutral scene where they were presented with a room without any spiders in order to optimise the HMD and get familiarised with the virtual environment. After all final adaptations were completed the neutral scene was again shown for 3 min directly followed by the fear-induction scene where 3 giant spiders, one of which seemed to move around the participants' feet, appeared in the office for an additional 3 min. As the VR confrontation was not intended to serve any therapeutic purpose, but was merely used to induce fear after the iTBS intervention, the participants were not given any instruction.

3.2.3.7 Data preperation

During the fNIRS measurement, changes in the concentration of O2Hb as well as HHb were registered from baseline. Subsequently, the HHb signal was chosen for further analysis as it is assumed to be less susceptible to stress-induced skin blood flow changes in the forehead (Sato et al., 2013). It was hence pre-processed with Matlab (The MathWorks, Inc., U.S.A.) in order to adjust the signal parts which were not associated with the attended task. This procedure included the following steps: application of a band pass filter to remove frequencies below 0.01 Hz and above 0.5 Hz, interpolation (<1% of all channels, as performed e.g. by Hagen et al. (2014) of noisy channels by corresponding HHb values of the remaining channels according to a Gaussian distribution (circumjacent channels were considered more than distant ones) and rejection of trials that clearly displayed technical artefacts after careful visual inspection by an experienced fNIRS researcher.

Finally, the data of each participant was segmented in an event-related manner for each channel, whereby a time frame of 0–16 s after stimulus onset was extracted and adjusted for linear drifts and baseline. Next, a general linear model was applied, wherein the data was modelled as $Y = \beta*X + \varepsilon$, with Y being the time × channel matrix comprising the fNIRS time series, β being the estimated parameter vector of beta-weights needed to model X, X being the design matrix containing the respective modelled effects, and ε describing the error term. The adopted function was a

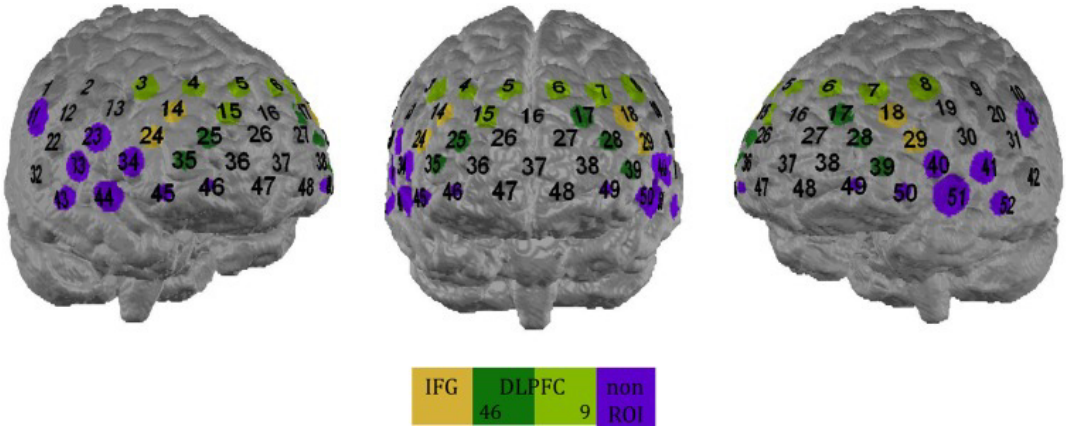
standard model used in SPM 8 (http://www.fil.ion.ucl.ac.uk/spm/doc/) as a haemodynamic response function (HRF) in terms of a gamma curve. While traditionally used for the analysis of fMRI data (Friston et al., 1995), this approach could also be validated for the analysis of fNIRS data (Plichta, Heinzel, Ehlis, Pauli, & Fallgatter, 2007). Following careful inspection of the averaged event-related HRF, a peak time of 8 s after stimulus onset was taken as the basis for the modelled curve. In order to estimate the beta weights, the method of least squares was used.

For the functional connectivity analysis, we refrained from the interpolation procedure in order to avoid spurious correlations between different brain regions.

3.2.3.8 Regions of interest (ROI)

In order to analyse oxygenation changes induced by the emotional stroop paradigm, different ROIs as well as a "non-ROI" were defined. To do so, first the regions lying under the probe set were mapped according to (Lancaster et al., 2000; Rorden & Brett, 2000; Singh et al., 2005; Tsuzuki et al., 2007). Based on the findings concerning regions that are particularly involved in cognitive control during the processing of emotional words (Dresler et al., 2012; Straube et al., 2004) the channels corresponding to the DLPFC (Brodmann area 9 and 46) as well as the bilateral IFG (Brodmann area 45) were defined as the main ROIs (see Fig. 1). Channels which repeatedly displayed muscle artefacts due to their spatial location close to the frontalis and temporoparietalis muscles were considered a non-ROI in order to exclude them from the Gaussian interpolation procedure.

Figure 1



Probeset arrangement with numbers indicating channels within the ROIs.

3.2.3.9 Statistical analyses

For each ROI 4 × 2 × 2 × 2 repeated measurement analyses of variance (RM-ANOVAs) were conducted for the corresponding β-weights whereby the within-subject factors were time (t0 versus t1) and condition (neutral versus phobic versus positive versus negative words) and the between-subject factors were group (phobics versus controls) and stimulation (verum versus sham). A Bonferroni-Holm (BH) correction procedure was applied to adjust the lowest alpha levels to $0.017 < p < 0.05$ per hemisphere in order to avoid alpha error accumulation due to the multiple analyses caused by the three different ROIs. For significant main or interaction effects, paired and independent two-tailed *t*-tests were employed for further post-hoc analysis.

In addition to the NIRS data, the complementing behavioural data, namely RTs and error rates, were analysed by means of corresponding repeated measurement ANOVAs. Regarding error rates, post-hoc testing was achieved by means of Mann-Whitney-*U* or Wilcoxon tests, as the data were not distributed normally.

Finally Pearson's correlation coefficients were calculated only for ROIs that displayed significant task-related HHb changes and corresponding RTs.

With respect to valence and arousal ratings RM-ANOVAs were performed once more, and post-hoc testing was achieved via paired and independent two-tailed *t*-tests in

the case of valence ratings and two-tailed Mann-Whitney-*U* and Wilcoxon tests in the case of arousal ratings to account for the deviation from normal distribution.

Finally, to further clarify our findings, a functional connectivity analysis (for similar fNIRS-based analyses refer to Medvedev, Kainerstorfer, Borisov, & VanMeter (2011)) was performed with the left IFG as the seed region. To do so, cross-correlation coefficients with a 0 time lag between the averaged HHb event-related time course of the left IFG and all other ROIs were calculated for the first 12 s after stimulus onset for each condition and time in a subject-wise manner. Before computing mean correlation values for each group, Fisher's z-transformation was applied to account for the fact that correlation coefficients are not interval scaled. In a final step a $4 \times 2 \times 2 \times 2$ RM-ANOVA with the same within- and between-subject factors as before, as well as the corresponding post-hoc *t*-tests, were performed. The alpha level was set to $p < 0.025$ in order to take the testing between the two hemispheres into consideration.

Due to motion artefacts during the fNIRS measurement which were too severe to be corrected (n = 9) as well as insufficient knowledge of the German language (n = 1) some of the subjects had to be excluded from the final analysis of the fNIRS data, resulting in a sample of n = 19 sham as well as n = 16 verum stimulated controls and n = 19 sham as well as n = 17 verum stimulated participants with spider phobia. Regarding behavioural data additional to the participant with insufficient German knowledge, one sham-stimulated participant in each group had to be excluded as the behavioural reactions were not recorded properly. In the end, with respect to the subjective ratings relating to the n = 71 fNIRS datasets, n = 1 sham-stimulated participant of the control group as well as n = 2 sham- and n = 2 verum-stimulated phobic participants were excluded from valence rating analysis. Regarding the arousal ratings n = 4 sham- as well as n = 1 verum-stimulated participants of the control group and n = 5 sham- as well as n = 4 verum-stimulated participants of the phobic group were dismissed from the analysis, respectively, due to systematic mistakes in rating (e.g. confounding the configuration of the rating scales, which differed between valence and arousal ratings, too many missing items). In this way, the final sample size differed slightly depending on the analysed variables.

3.2.4 Results

3.2.4.1 Behavioural performance

In order to verify whether implicit emotion regulation during the confrontation with fear-inducing stimuli is impaired in participants with spider phobia, RTs and error rates were investigated. Hereby we expected an increase in behavioural performance after the VR challenge, particularly in the verum-stimulated group (hypothesis 2 and 3).

In terms of RTs (for a complete overview of mean RTs please refer to Table 2 at the end of the section) the emotional-word Stroop task revealed a significant main effect for the factor condition ($F3195 = 3.91$, $p < 0.001$) whereby the reaction to negative words was generally slower than to all other conditions (RTs neutral: 654+/−111 ms; RT positive: 653+/−111 ms; RTs phobic: 652 +/−118 ms; RTs negative: 664 +/−120 ms). There were no other significant main effects or interactions.

Concerning error rates (for a complete overview of mean error rates please refer to Table 3 at the end of the section), there was a significant main effect of the factor time ($F1,65 = 10.88$, $p = 0.002$), indicating that more errors were committed at t1 (errors t0: 0.71+/−0.67; errors t1: 0.9 +/−0.6). Moreover, there was a significant interaction of time*group*stimulation ($F1,65 = 6.63$, $p = 0.012$) which could be explained by an increase in committed errors from t0 to t1 especially in the group of sham-stimulated controls ($z = −1.969$, $p = 0.04$) and the group of verum-stimulated phobic participants ($z = −2.163$, $p = 0.031$). Pairwise group comparisons however, did not reveal any significant differences. All in all, we did not find an increase in behavioural performance after iTBS and neither an impaired performance in participants with spider phobia compared to healthy controls.

Table 2

	participants with spider phobia – verum iTBS n=				Participants with spider phobia – sham iTBS n=				Healthy controls – verum iTBS n=				Healthy controls – sham iTBS n=			
t0	neutral 647 (99)	phobic 654 (99)	negative 657 (97)	positive 654 (107)	neutral 641 (131)	phobic 651 (139)	negative 669 (161)	positive 659 (145)	neutral 645 (141)	phobic 633 (146)	negative 650 (144)	positive 637 (132)	neutral 621 (89)	phobic 641 (110)	negative 662 (122)	positive 627 (95)
t1	643 (117)	623 (109)	637 (113)	631 (101)	661 (153)	655 (168)	658 (155)	664 (151)	646 (126)	655 (121)	662 (137)	648 (121)	651 (96)	641 (90)	648 (83)	642 (89)

Table 2. Mean reaction times and standard deviation (in brackets) for all conditions and groups shown separately for t0 and t1.

Table 3

	participants with spider phobia – verum iTBS n=				Participants with spider phobia – sham iTBS n=				Healthy controls – verum iTBS n=				Healthy controls – sham iTBS n=			
t0	neutral 0.88 (1.32)	phobic 0.47 (0.72)	negative 0.88 (0.99)	positive 0.76 (0.97)	neutral 1.00 (1.33)	phobic 0.78 (1.48)	negative 0.67 (1.24)	positive 0.78 (1.00)	neutral 0.81 (0.91)	phobic 0.88 (0.72)	negative 0.81 (0.91)	positive 0.81 (0.91)	neutral 0,72 (0.83)	phobic 0.50 (0.71)	negative 0.50 (0.62)	positive 0.39 (0.85)
t1	1.47 (1.18)	1.24 (0.90)	1.18 (1.29)	1.12 (0.78)	0.67 (0.77)	1.06 (1.06)	0.89 (1.18)	0.83 (0.79)	0.94 (0.93)	0.88 (1.14)	0.75 (0.93)	1.00 (1.16)	0.67 (0.68)	1.00 (1.14)	1.22 (1.00)	1.06 (0.94)

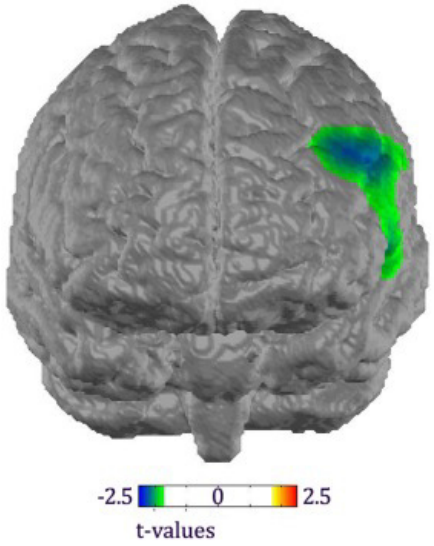
Table 3. Mean error rates and standard deviation (in brackets) for all conditions and groups shown separately for t0 and t1

3.2.4.2 Cortical activation

In terms of cortical activation patterns, we hypothesised increased DLPFC/IFG activation in participants with spider phobia due to higher demands on cognitive control when responding to phobia-related stimuli. We further expected that VR in combination with the activating rTMS protocol would result in an additional increase in activation, in this context reflecting a higher recruitment of prefrontal resources for the execution of the task (hypothesis 1 and 3).

With regard to the left IFG, there was a significant interaction of time*group ($F_{1,67} = 7.73$, $p = 0.007 < 0.017$, BH-corrected) which was accounted for by reduced activation in the phobic compared to the control group at t0 ($t_{69} = -2.22$, $p = 0.03$; see Fig. 2) but not at t1. When comparing changes in cortical oxygenation over time, a significant decrease could only be found in the control group ($t_{34} = 2.54$, $p = 0.016$). Even though the activation in the phobic group increased from t0 to t1 on a descriptive level, this difference was not significant.

Figure 2



HHb concentration levels contrasted between control and phobic participants at t0 by means of t-values for each channel. Only significant values are presented.

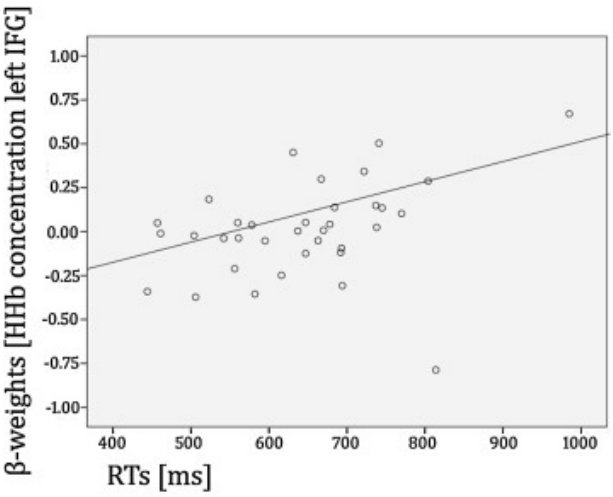
Concluding, contrary to our hypothesis, we found reduced rather than increased PFC activation in participants with spider phobia compared to healthy controls. An explanation for this finding will be discussed. Moreover, in line with what we expected, there was an increase in activation after the VR iTBS combination at least on a descriptive level. Verum iTBS did not cause an additional effect, however.

3.2.4.3 Correlation of behavioural performance and cortical activation

Inherently, we expected an association between brain activation and behavioural performance in terms of significant correlations between RTs/Error rates and HHb concentration (hypothesis 1 and 2).

However, a significant correlation could only be found between RTs and HHb concentration (β weights) in the left IFG in the phobic group for neutral words at t0($r = 0.355$, $p = 0.017$; see Figure 3).

Figure 3

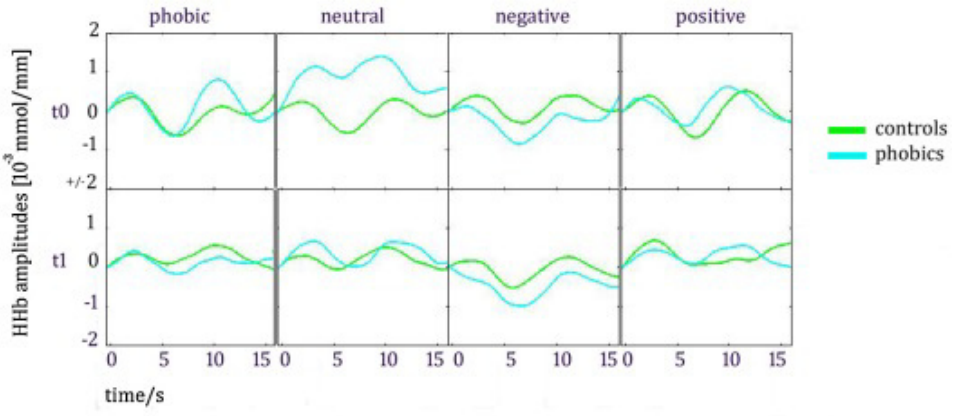


Scatterplot RTs and cortical HHb concentration in the left IFG at t0 for neutral word in the group of participants with spider phobia.

To get a better idea of how to interpret this finding, we looked at the averaged haemodynamic response functions (HRF) for each condition and measurement time

in the phobic and the control group separately. Strikingly, on a descriptive level, mean HHb concentrations were especially high (inferring reduced activation) for neutral words in the phobic group at t0 as depicted in Figure 4.

Figure 4



Averaged HRF of HHb at t0 and t1 for each condition in the phobic and control group, separately. According to the model-based analysis there was a significant difference between the two groups at t0.

3.2.4.4 Valence and arousal ratings

With respect to the subjective ratings of the presented words, we generally intended to replicate our results from the pilot study in order to show that our experimental manipulation was successful (graduation of valence and arousal ratings according to the specific word category without a difference between groups except for the phobic words). Following VR, we further postulated a temporary assimilation between the group of participants with spider phobia and controls, regarding subjectively perceived valence and arousal ratings of the phobia-relevant words. This effect was assumed to be bigger in the verum-stimulated group (hypothesis 3).

Concerning valence ratings of the presented words, a main effect for condition ($F_{3186} = 1248.48$, $p < 0.001$) was detected, indicating that the valence for negative words was rated < the one for phobic words < the one for neutral words < the one for positive words (all $p < 0.001$). Furthermore, there was an interaction of condition*group ($F_{3186} = 37.40$, $p < 0.001$) since, as expected, phobic participants

judged the phobic words as more negative than the control group ($t55 = 11.54$, $p < 0.001$). An interaction of condition*time ($F1,26 = 7.74$, $p = 0.003$) revealed that while ratings for neutral and phobic words were stable over time, ratings for the negative ($t65 = -4.00$, $p < 0.001$) and positive words ($t65 = 2.18$, $p = 0.033$) were more negative at t1. Finally, there was a significant interaction of condition*group*stimulation ($F1,84 = 5.64$, $p = 0.009$). This interaction was basically due to the fact that (as for the entire group) the only difference in valence rating between the phobic and control group was found for phobic words in the verum-stimulated group ($t18 = -9.30$, $p < 0.001$), while in the sham group a significant difference arose not only for phobic words ($t34 = -7.31$, $p < 0.001$) but also for negative words (which were rated more negative in the control group ($t25.82 = 2.14$, $p = 0.042$).

In line with the valence ratings, a main effect of condition ($F2113 = 125.43$, $p < 0.001$) was also detected for the arousal ratings whereby the arousal for emotionally positive and emotionally negative words did not differ significantly but was > the arousal for phobic words > the one for neutral words (all $p < 0.001$). Accordingly, there was also an interaction of condition*group ($F2113 = 17.27$, $p < 0.001$) whereby the pairwise group comparison revealed significantly higher arousal ratings for phobic words solely in the group of spider phobic participants ($z = -4.677$, $p < 0.001$). In addition, in the phobic group only, the difference in arousal ratings between the emotionally negative and positive control words and the phobic words was not significant. An interaction of time*stimulation ($F1,53 = 5.67$, $p = 0.021$) further indicated that the arousal ratings in the sham-stimulated group only decreased from t0 to t1 ($z = -2.35$, $p = 0.019$). Aside from that, there was again an interaction of condition*group*stimulation ($F2113 = 5.337$, $p = 0.005$) whereby, equivalent to the valence rating, the only significant difference between the phobic and control group occurred for phobic words in the verum group ($z = -3.69$, $p < 0.001$). In the sham-stimulated group, however, the group differences were not only significant for phobic words ($z = -2.62$, $p = 0.009$) but also for negative ($z = -2.62$, $p = 0.017$) and positive ($z = -1.99$, $p = 0.046$) words, for which – contrary to the phobic words – the arousal was lower for the emotional control words in the phobic group.

Finally, there was an interaction of condition*time*group ($F2133 = 7.25$, $p < 0.001$) that was mainly carried by different changes in arousal ratings for the control and phobic group. Whereas in the control group ($z = -2.55$, $p = 0.01$) as well as in the phobic group ($z = -1.98$, $p = 0.048$) the arousal for neutral words increased from t0 to t1, the arousal for phobic words solely decreased in the phobic group ($z = -3.99$, $p < 0.001$).

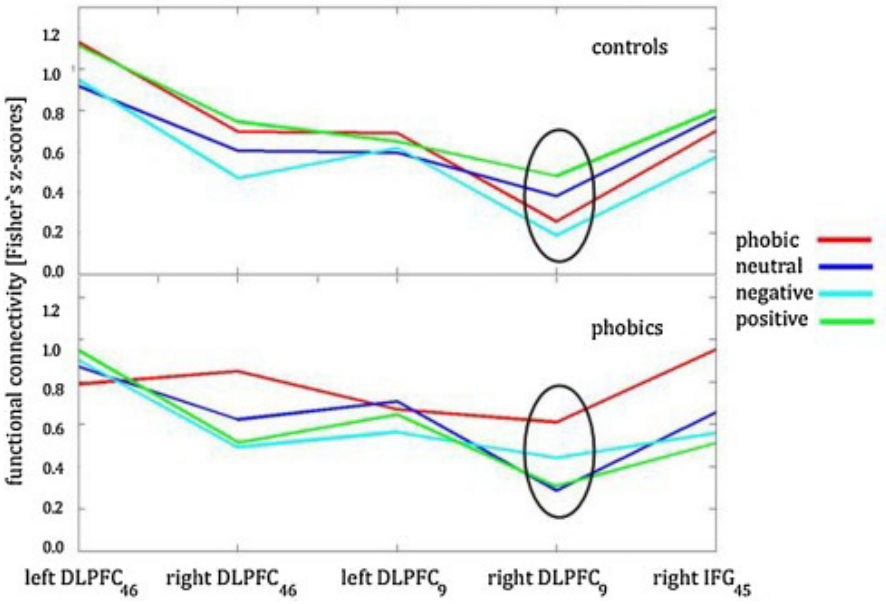
Hence, in summary, the evaluation of the subjective ratings confirmed a successful experimental manipulation in terms of significant group differences between participants with spider phobia and healthy controls regarding valence and arousal ratings of phobic words explicitly (replicating the results from our pilot study as expected). Furthermore, there was a significant decrease with respect to the rated arousal for phobic words from t0 to t1 only in the group of participants with spider phobia which was also in line with our assumption. But in contrast to hypothesis 3, this effect was not bigger in the verum-stimulated group.

3.2.4.5 Functional conectivity of the left IFG

Finally, we performed a functional connectivity analysis in order to get a better understanding of the interaction of different sub-regions within the PFC during emotional control processes (hypothesis 4). Functional co-activation was thereby defined as the cross-correlation of the mean HHb time course for each condition between the left IFG and all other ROIs (ipsilateral DLPFC9/46 as well as contralateral DLPFC9/46 and contralateral IFG). As a result, we found a significant interaction of condition*group ($F3201 = 3.33$, $p = 0.021 < 0.025$, BH-corrected) for the right DLPFC9. In this context, the functional connectivity was increased in the phobic compared to the control group for phobic words only ($t69 = 1.96$, $p = 0.05$). Moreover, the within-group comparisons indicated a significant difference in co-activation patterns for the control group solely when contrasting emotionally negative and emotionally positive words, wherein the connectivity was decreased for negative words ($t34 = 2.13$, $p = 0.041$). On the other hand, in the group of phobic participants the co-activation between left IFG and right DLPFC9 was significantly

enhanced for phobic compared to neutral (t35 = 2.472, p = 0.018), as well as phobic compared to positive, words (t35 = 2.604, p = 0.012; see Figure 5).

Figure 5



Functional co-activation of the left IFG and all other ROIs for each condition in the control as well as the phobic group at t0. Higher numbers of Fisher's z-score indicate increased functional connectivity.

3.2.5 Discussion

The aim of this study was to verify whether spider phobia is characterised by alterations in prefrontal brain activation patterns, as assessed by fNIRS when being confronted with fear-inducing stimuli, in terms of an emotional Stroop paradigm. In a second step, we applied a one-time sham-controlled iTBS protocol over the left PFC which was followed by a spider challenge in a virtual environment that served as a triggering situation to elicit anxiety. The participants were not given any therapeutic instructions, as we merely wanted to evaluate the neural (fNIRS) and behavioural (RTs, error rates, valence, arousal) impact of iTBS in combination with a fear-relevant situation without further confounding effects of therapist-guided exposure. In addition to a standard fNIRS analysis of cortical activation patterns, we also conducted a functional connectivity analysis in order to get a better understanding of our results.

Contrary to our first question, we found reduced activation in the left IFG of participants with spider phobia as compared to healthy controls at t0.

With respect to our second hypothesis, we did not find reduced performance in terms of slower RTs or increased errors rates in the group of phobic participants at either t0 or t1 compared to healthy controls. However, a significant correlation between the RTs for neutral words and activation in the left IFG could be detected solely for phobic participants at t0. At the same time, RTs were generally significantly reduced for negative words in the entire group of participants. Regarding the third question, we found that after the iTBS/VR combination, the difference in prefrontal activation disappeared. However, this adjustment was independent of the stimulation group (verum versus sham). The valence and arousal ratings generally confirm that the emotional categories of our Stroop paradigm were perceived as intended. In this context, the valence of negative words was overall judged as the most unfavourable followed by phobic words while neutral words were located in the middle of the Likert scale. Positive words were perceived as most favourable. Regarding arousal ratings, the arousal for emotionally negative and emotionally positive words was the highest in the entire group of participants, followed by phobic and then neutral words. Group differences between phobic and control participants were significant with respect to explicitly phobic words—as expected, spider phobic participants rated phobic words as more negative, thereby indicating a higher arousal. Furthermore, there was a significant effect of time in both groups, whereby the emotional control words were judged as more negative in the second rating session. Concerning both valence and arousal ratings, there were differences in ratings for the separate word categories between the sham- and verum-stimulated groups which differed between controls and phobic participants. However, as these differences between groups did not depend on the rating session (prior to, versus after, the iTBS application; i.e., they were already present at the baseline assessment) they are unlikely to represent real stimulation effects but rather suggest that group sizes should have probably been slightly bigger in order to achieve a better control of random assignment effects. Regarding these baseline differences between the sham- and verum-stimulated group, it is very likely that the same effect also accounts for the decrease in arousal after iTBS in the sham-stimulated group only. Interestingly, however,

comparable to the alterations in neuronal activation, changes regarding the rating scales over time were independent of the stimulation, but pointed towards an adjustment of the phobic to the control group (decrease in arousal for phobic words exclusively in the phobic group). Moreover, there was a significant change in arousal over time, indicated by an increase for neutral words in phobic and control participants. On a behavioural level this increase in arousal was accompanied by an increase in errors at t1, which was independent of the word category, probably pointing to a decrease in motivation and/or concentration.

With respect to our forth question, we observed a significant increase in co-activation of the left IFG and the contra-lateral DLPFC in the phobic compared to the control group. Strikingly, this increase was characterised by enhanced connectivity for exclusively phobic words in the phobic group as compared to the control group. Additionally, the coherent activation was significantly decreased for negative as compared to positive words in the control group, and significantly increased for phobic relative to neutral as well as positive words in the group of spider phobics.

Overall, it is not surprising that the detected differences in cortical activation were only significant in the left IFG, as this region comprises Broca's area and is hence most important for the processing of semantic stimuli as in the case of an emotional-word Stroop paradigm. Furthermore, unlike in other studies (Dresler et al., 2012) that additionally reported activation differences in other prefrontal areas, the behavioural performance of participants with spider phobia was not at all impaired, strongly pointing to a compensating mechanism. In line with this assumption, we investigated the functional connectivity of the left IFG with the other ROIs (ipsilateral DLPFC9/46 as well as contralateral DLPFC9/46 and contralateral IFG), hypothesising that we should find increased co-activation in the group of participants with spider phobia, which could explain the preserved performance level regarding phobic words. In line with this hypothesis, we were able to confirm a significant augmentation of coherent activation of the right hemisphere for the group of participants with spider phobia in response to phobic words. Even though only significant for the right DLPFC Brodmann area 9, on a descriptive level, this increase in functional connectivity could also be observed for the other ROIs of the contra-lateral hemisphere (compare Fig. 5) underlining the interpretation of a compensatory

effect. At first glance it seems counter-intuitive that healthy controls should display a relative decrease in connectivity for negative words when at the same time phobic participants are characterised by an increase in connectivity for phobic words. However, it should be considered that the context of the experiment certainly also plays an important role. While the phobic participants were prepared from the beginning to respond to fearful stimuli, the situation was in general neutral for the control group. Moreover, it also needs to be kept in mind that, although not intended, parts of the control group were defined by lower valence and higher arousal ratings for negative words. Finally, when looking at the entire group, the performance level could not be maintained for negative words. Again, valence ratings were generally the lowest accompanied with a high arousal which further validates the idea of a (in this case insufficient) supportive prefrontal network.

In conclusion, it can be assumed that fearful situations elicit the recruitment of a prefrontal compensatory network in order to allow for the down-regulation of emotional reactions and hence adequate behavioural reactions. If the fear triggered by adverse stimuli reaches a certain level, however, this network breaks down, leading to decrease in performance. Within this context, the fact that the phobic participants did not necessarily need to experience constraints in their daily routine might also serve as an explanation why there were no differences regarding behavioural performance. At their level of anxiety, compensation via the recruitment of other prefrontal areas was probably still possible. If we had only included people which were truly limited by their fear of spiders in their everyday life, it is likely that the network would not have offset the perception of phobic stimuli any longer, resulting in a decrease in performance.

Regarding the activation within the left IFG itself, we found a general decrease in activation in the phobic group compared to the control group. This was surprising at first, as other studies have reported enhanced activation to emotionally relevant stimuli in Broca's area (Dresler et al., 2012; Straube et al., 2004). Interestingly, however, this effect was mainly carried by decreased activation to neutral words in the phobic group at t0. Even though there was no significant interaction with the factor condition, when looking at Fig. 4, it is striking that the HRF is especially reduced for this condition. Moreover, a direct correlation between behavioural

performance and activation in the left IFG was found exclusively for this word category at t0 for phobic participants. This observation suggests an interpretation in terms of the already discussed network hypothesis: presumably neutral words carry the least emotional relevance in a potential phobic situation and hence receive the least support by compensatory co-activation of other prefrontal areas, on the one hand leading to the generally lowest cortical activation, but on the other hand leading to a more direct relationship between this activation and the visible behavioural performance. Although this idea is of course rather speculative, it can account for all our findings, and seems therefore worthy of further investigation examining this hypothesis in more detail.

On a final note, we could not confirm a modulatory effect of iTBS on either cortical activation, behavioural performance or perceived emotional content of the stimuli. A possible explanation for this finding might be the time delay between iTBS application and the second fNIRS measurement (t1). As mentioned in the introduction, past studies mainly found acute effects of iTBS which lasted up to approximately an hour (Grossheinrich et al., 2009). In the present study, however, the average time delay was over an hour, due to the VR challenge and associated preparations succeeding iTBS. Even though this certainly represents a major limitation of our study, the fact that we could not find any physiological changes in terms of alterations in heart rate or skin conductance and respectively perceived disgust and anxiety (these results of the same study sample have been reported in section 3.1) during the VR makes it unlikely that our null findings are only due to methodological reasons. Another explanation for the missing iTBS effect may be the state-dependency of rTMS which basically infers that, depending on the already ongoing brain activity, the effects of rTMS may significantly vary between subjects (Silvanto & Pascual-Leone, 2008). However, in this context a review article by Sandrini, Umiltà, & Rusconi (2011) suggests that an activating protocol may specifically affect neural populations which display the lowest activation prior to stimulation onset. Therefore, we should have expected at least a trend-wise enhancement of neural activation in the verum-stimulated group of participants with spider phobia for specifically neutral words. It would probably still be premature to deduce that iTBS can generally not serve as a supportive tool with respect to

emotion regulation in fearful situations from our results. Instead, future studies should try to eliminate even more confounding factors in order to get a better understanding of its possible mode of action. Although we attempted to exclude other active factors by not giving any therapeutical instruction prior to the VR challenge, our results point to a generally fear-reducing effect triggered by mere confrontation with the virtual spiders. This reasoning is supported by the adjustment of cortical activation patterns at t1 as well as the significant decrease in perceived arousal in the phobic group. Another minor factor which should still not be neglected when interpreting our findings is the apparent decrease in performance from t0 to t1 in the entire group of participants as indicated by the increase in errors as well as the changes concerning word ratings (e.g. the enhanced arousal to neutral words at the second rating session). A probable explanation for this might be a decline of motivation and/or concentration in the context of the long duration of the study. Such restricting factors could have further interfered with potential iTBS effects, which might have been detectable otherwise. Finally, within the framework of a combination of rTMS and VR it must be kept in mind that the *feeling of presence* – defined as the impression of actually being in the particular environment (Slater, 1999) – is significantly modulated by a prefrontal network including the DLPFC as a pivot point, whereby the relationship between prefrontal activation and the feeling of being there is a negative one (Jäncke, Cheetham, & Baumgartner, 2009). This correlation could not only be replicated in a multitude of studies investigating patients with post traumatic stress disorder during dissociation (Hopper, Frewen, Van der Kolk, & Lanius, 2007; Lanius et al., 2010; Lanius et al., 2002) but could furthermore be found in healthy control subjects during VR immersion (Baumgartner et al., 2008). Accordingly, in our study we may have induced two counteracting effects by the application of iTBS: on the one hand the activating verum stimulation might have led to more efficient emotion regulation in terms of better cognitive control, while on the other hand, by doing so, the *feeling of presence* in the virtual scenario could have been diminished, resulting in a fainter (learning) experience. This could explain the missing effects on our dependent variables at t1, including the ratings of the emotional stimuli. These antagonising stimulation effects represent a further limitation with respect to our study design. In line with the results of Beeli,

Casutt, Baumgartner, & Jäncke (2008) we could, however, not confirm a significant effect of verum iTBS on the perceived *feeling of presence,* but rather only on the activation of the vegetative nervous system (section 3.1). Nevertheless, despite a large number of studies, there is no conclusive evidence for a direct relationship between initial (physiological) fear activation and a positive outcome of exposure therapy (Craske et al., 2008). Future investigations should therefore try to unravel the impact of iTBS into its separate components and then further explore its influence on the effectiveness of exposure therapy.

3.2.6 Conclusion

The results of the present study showed a generally diminished activation (which was particularly pronounced for neutral words) in response to semantic stimuli in the left IFG during a phobia-relevant emotional Stroop paradigm in the phobic group compared to a healthy control group. This decrease in activation correlated positively with behavioural performance, in terms of RTs, solely for emotionally irrelevant words and was thereby associated with a particular distinctive activation decline. Interestingly, behavioural performance was generally not impaired, which could be explained in terms of a compensatory prefrontal network that supports the maintenance of adequate behavioural responses in fear-relevant situations. If the adverse emotional response triggered by the situation succeeds a certain threshold, however, this network breaks down, and performance decreases, as was the case in response to negative words in our study. Apart from these findings, we found an adjustment of the reported alterations in the left IFG in phobic participants compared to healthy controls after a confrontation with virtual spiders, which was accompanied by a significant decrease on arousal ratings of the phobic words. This effect was independent of preceding iTBS application, on the one hand supporting the potential of virtual scenarios as part of exposure therapy, on the other hand challenging the application of single iTBS sessions in the context of emotion regulation.

4. General discussion

4.1 Summary and conclusion

The present work has been dedicated to the investigation of iTBS as a qualified treatment option in the therapy of anxiety disorders. The theoretical background to this research question was the imbalance of the fear network, characterised by prefrontal hypoactivation and hyperactivation of e.g. the amygdala which has been repeatedly reported in the literature (see section 1.3.1). Moreover, the ability of rTMS and, within this context iTBS, to distinctly modulate cortical activation patterns in a non-invasive manner has been demonstrated in multiple studies as well as clinical case reports before (see section 1.4.2.4). Drawing the conclusion from these findings, we designed two studies, whereby each addressed a different possible application of iTBS in the treatment of anxiety disorders. Hence, the first study examined the effects of repeated (sham-controlled) iTBS administration as an add-on tool during the time course of standardized CBT (15 sessions conducted during the first three weeks) in a group of patients suffering from panic disorder with or without agoraphobia. The second study on the other side, focused on the one-time iTBS effect on anxiety symptoms (subjectively perceived fear as well as behavioural and psychophysiological symptoms) prior to a fear-inducing situation in a group of spider phobic subjects. In both studies, prefrontal activation was recorded before as well as after iTBS treatment by means of fNIRS and finally compared to the prefrontal activation patterns of a healthy control group.

In general, both studies could (1) replicate alterations within the fear network in terms of divergent prefrontal activation patterns compared to healthy controls. (2) Further, these deviations in prefrontal activity could partly be normalised after iTBS application. (3) However, clinical effects, in terms of a subjectively improved symptom reduction after verum iTBS could not be demonstrated in either of the studies. The following sections will discuss these results across both studies in more detail and finally draw conclusions for possible clinical applications.

4.2 Integration of finding (1) in terms of altered prefrontal activation in anxiety disorders into the current literature

In line with the current literature, as stated in the introduction, both our studies could confirm alterations within the fear network in terms of altered prefrontal activation. In more detail, study 1 revealed bilateral prefrontal hypoactivation in patients with panic disorder compared to healthy controls during a cognitive task as well as left sided hypoactivation in response to panic-relevant stimuli during an emotional Stroop paradigm. Study 2 on the other hand, showed left-sided hypoactivation of the IFG in spider phobic participants elicited by emotionally irrelevant words during a Stroop paradigm. Even though at first sight, these results seem to be conflicting in terms of the elicited activational differences, they can be integrated when including further data as the functional connectivity analysis, or behavioural data into the interpretation. In this regard, it needs to be kept in mind, that the behavioural performance of patients suffering from panic disorder was impaired, whereas there was no decline in performance for the group of spider phobic participants. At the same time, the functional connectivity analysis that was conducted in study 2 pointed to a compensatory network whereby contralateral resources may be recruited in order to maintain adequate behavioural reactions as long as possible. Within the framework of this idea, it may be concluded that such additional resources might rather be distributed to the processing of emotionally relevant stimuli thereby explaining the relative hypoactivation towards emotionally irrelevant stimuli. In fact, this perspective may also serve as an explanatory approach for the diverging literature findings with respect to the valence hypothesis: whereby on the one hand, this model of a lateral asymmetry regarding the valence of affective processing could be replicated in many studies (see 1.3.2), on the other hand there have also been results which showed bilateral hypoactivation (Ball, Ramsawh, Campbell-Sills, Paulus, & Stein, 2013) in different groups of patients or even on the contrary, increased activation towards phobic stimuli (Schienle et al., 2005; Straube, Mentzel, & Miltner, 2006). Surely, these diverging results may also be due to a number of different factors including the chosen presented affective stimuli or study populations, yet it may be worthwhile to investigate this point of view in a study which specifically manipulates the emotional content and associated

maintenance of behavioural response while assessing the functional connectivity across hemispheres. Besides the hypothesised prefrontal hypoactivation in response to emotional stimuli, we also found bilateral hypoactivation during a cognitive task (verbal fluency task) in our group of patients with panic disorder which replicates the results of Nishimura et al. (2007) and underlines the idea that the imbalance of the fear network found in affective disorders is not restricted to emotional processing, but rather represents a more general trait across situations (Sylvester et al., 2012). Even though it needs to be kept in mind that in our study the fNIRS recording situation itself may have presented a fear-relevant situation for patients with panic disorder, hence explaining the detected hypoactivation, the fact that these aberrant activational patterns were only found during the experimental and not during the control condition speaks against such an assumption. In fact, the findings may even be integrated into the above presented idea as an example of why the generally hypothesised left-sided hypoactivation in anxiety disorders may not be found depending on the experimental condition. In this case, our paradigm presented a task based on language production which would be expected to cause increased activation in the left hemisphere (Vigneau et al., 2006) which could indeed be shown when comparing hemispheric differences between the experimental and control condition. Thus, it would make sense that a language-based paradigm, which does not primarily aim at the processing of affective stimuli, may result in bilateral prefrontal hypoactivation by "hiding" the more pronounced left-sided deficit due to the activation elicited by language processing. All in all, together our studies could confirm that there are changes in prefrontal activation patterns which seem to be detectable for different anxiety disorders as well as across different experimental conditions (fear-specific versus general) and thus seem to present a rather general trait of anxiety disorders. Yet, the manner in which these alterations of the fear network present themselves probably depends on the specific situation whereby particularly the difficulty of the task and hence the recruitment of a compensatory network to hold up the performance level may play an important role. Last but not least, we could show that fNIRS is a valid tool to collect such data including sufficient information to conduct a functional connectivity analysis of the prefrontal cortex (Medvedev et al., 2011) despite prior findings which suggested that the data quality

over prefrontal areas is rather poor (as compared to other regions) due to the anatomical characteristics of the area (e.g. frontal sinuses (Haeussinger et al., 2011)). In this regard, the present work can finally also confirm the usefulness of fNIRS to address further questions focusing on the interplay of different prefrontal areas rather than the isolated investigation of specific regions of interest, which may be more and more important in the future.

4.3 Finding (2): the effects of iTBS on psychophysiology and prefrontal activation patterns

Regarding iTBS application, we could not obtain consistent findings. While in study 1 we found significant increases in bilateral prefrontal activation solely after the completion of 15 verum iTBS sessions in response to panic-relevant stimuli, there were no such changes during the cognitive paradigm. In fact, contrary to our expectation, a significant increase of brain activation in the left hemisphere was solely found in the sham-stimulated group. Study 2, on the other hand, could not confirm any effects on brain activation after the single iTBS session. However, significant changes in HRV (but neither HR per se nor EDA) after verum stimulation could be reported. First of all, looking at these opposing findings on brain activation (significant increase in neural activation after verum iTBS versus significant increase after sham iTBS versus no significant increase at all), an explanation that comes to mind straight away is that one session is simply not sufficient at least when regarding the missing effects after the one-time application in study 2. Yet, there are a number of prior studies that repeatedly showed a robust effect on brain activation after one session (Huang et al., 2005; Tupak, Dresler, et al., 2013). Surely, another reason why, in contrast to other studies, we could not find any acute effects of iTBS might further be given by our experimental set-up. Despite the fact that our VR challenge was supposed to only serve as a fear-inducing situation without any therapeutical guidance, the sole exposure to the phobic objects may still have already been enough to cause some kind of "habituation" effect and accordingly an adjustment in brain activation in both, verum and sham-stimulated group hence

resulting in a ceiling effect. Still, it makes sense to also take the results from study 1 into consideration and try and integrate both findings. To begin with, in our first manuscript (see section 2.1) we discussed that the increase in brain activation after sham stimulation was probably due to either a more general arousal related effect or else regression to the mean since the patients in the sham-stimulated group actually showed reduced activation compared to the verum-stimulated group at the first measurement time. This is a valid conclusion, yet it does not explain why there was indeed a specific effect of verum iTBS on panic-related stimuli for the emotional Stroop paradigm. Moreover, one might ask why in study 2 we did not find an effect of verum iTBS in the group of healthy control subjects since in their case the exposure to virtual spiders should not have triggered a fear reaction and thus cannot be explained in terms of a ceiling effect due to an unintended therapeutic intervention. Taken together, an explanation which comprises all above depicted results can be given by the assumption that the impact of iTBS actually depends on the prior states of the underlying neuronal circuits which has already been shown previously (Silvanto & Pascual-Leone, 2008) and was therefore discussed separately in our manuscripts. However, in order to resume all our findings it is necessary to go into even more detail in order to draw a comprehensive conclusion for future possibilities of iTBS application. To do so, it may be interesting to go back in time and look at an earlier visual adaption experiment (Silvanto, Muggleton, Cowey, & Walsh, 2007) in which the authors manipulated the excitability state of specific neural populations by adaption (prolonged exposure to a sensory stimulus in order to influence the perception of a subsequent stimulus (Gibson & Radner, 1937)) to different optical stimuli before excitatory TMS application over the visual cortex which is known to induce phosphene sensations (O'Shea & Walsh, 2007). Summing it up, they found that TMS especially facilitates the perception of the stimulus properties represented by less active neural circuitries. Thus, this finding may be able to explain the missing iTBS effect for the cognitive paradigm of study 1 as well as for the emotional Stroop paradigm of study 2 regarding phobic subjects, just the same as healthy controls. In this regard, it can be assumed that in all cases the stimulated underlying neural populations were already rather excited (no prefrontal hypoactivation in healthy controls; additional recruitment of compensatory network

in spider phobics; specific activation of left-hemispheric prefrontal activation due to language-based cognitive paradigm, compare last section), so iTBS did not result in an additional effect. Moreover, it needs to be kept in mind that we observed a significant change in HRV in study 2 in the verum-stimulated group only. However, the change in HRV did not point to a decrease in activation of the sympathetic nervous system as we originally expected, but on the contrary suggested an increase. We interpreted this in terms of an increased attention towards the presented stimuli which can be seen as some kind of cognitive control mechanism which was possibly masked by compensatory activation on a neural level but still manifested itself on a psychophysiological level. However, in contrast to this, patients suffering from panic disorder already showed diminished prefrontal activation in response to panic-relevant stimuli at the baseline measurement. Hence, iTBS application met an under-activated neural brain state and could consequently induce an increase in prefrontal activation to panic-relevant stimuli, as we had hypothesised. A possibility to manipulate the neural *pre-state* in a standardised manner is to combine rTMS with tDSC. In fact, there are already a few sham-controlled studies using traditional rTMS (Lang et al., 2004) as well as TBS (Weigand et al., 2013) that investigated the integration of these two methods. So far, the conclusion which can be drawn from the results is that excitatory rTMS can induce the greatest activational increase when being preceded by an about ten minutes lasting cathodal tDCS application. When applied in this manner, verum rTMS is not only superior to sham stimulation but also exceeds the results of traditional rTMS treatment without prior tDCS application. Concluding from these findings, it may be indicated to first systematically manipulate the initial brain state before rTMS administration during prospective studies.

On a similar account, contextual as well as time-related factors probably also play an important role for the impact of the stimulation protocol, especially when assessing the anxiolytic effects of rTMS or more specifically iTBS. In this context, it may be reasonable to differentiate between the repeated applications of brain stimulation techniques such as iTBS or tDCS during the time course of psychotherapy, as we did in study 1 and single applications in combination with exposure as we did in study 2. For example, when administered during psychotherapy, besides the choice of the

stimulation protocol, it may be essential during which parts of the therapeutic process the stimulation takes place. This is even more important to consider as the different brainstimulation techniques do not only enhance or decrease neural activation on a short term basis, but are further able to trigger neuroplastic changes when applied repeatedly (see section 1.4.2.4). Thus, an important research question might be under which conditions a particular brainstimulation protocol should be applied before, after or even during (in case of sole tDCS) a therapy session. Within this framework, another concern could be whether there are specific therapeutic modules like the psychoeducational phase at the beginning, cognitive restructuring elements and emotional activation or resource-oriented working when the add-on effect might be of the most supportive use. Similarly, regarding the one-time use of brainstimulation techniques, more research is needed concerning the stimulation time as well as additional factors. Thus, in order to choose a fitting stimulation method, possible questions to pose might be: is it the main goal to enhance neuroplasticity for improved extinction learning? Should the patient achieve better cognitive control during a fear-inducing situation? Or may even the opposite effect in terms of an intensification of the fear-induction be desirable? Of course, the latter point might especially make sense when working with patients that tend to use cognitive avoidance as a rather automated strategy in order to evade fear activation.

Regarding this matter of an intensified fear-induction effect, it seems worthwhile to get back to study 2 where we combined iTBS with the VR scenario. Even though a number of studies (see section 1.4.2.3) including ours have shown that VR can trigger subjectively perceived fear as well as the corresponding physiological reaction, it cannot be assumed that all potential participants manage to equally immerse into the given VR scenario thereby experiencing an actual feeling of presence. At the same time, studies have found a significant negative correlation between DLPFC activation and the feeling of presence during VR immersion (Baumgartner et al., 2008) which is in accordance with the finding that patients suffering from posttraumatic stress disorder accompanied by derealisation or depersonalisation phenomena also show increased activation in prefrontal areas (Hopper et al., 2007; Lanius et al., 2010; Lanius et al., 2002). Thus, a possible application in terms of fear-induction during virtual VR therapy could be the down-

regulation of prefrontal activation prior to a VR challenge in order to enhance the feeling of presence. Surely, such considerations are still highly speculative at this point but nevertheless deserve further attention in prospective studies.

4.4 Further explanations for the missing clinical effects of prefrontal iTBS (finding 3)

Whereas on a neurobiological level we could show some significant iTBS induced changes in prefrontal activation and HRV, on a clinical level no differences in terms of phobic symptom reduction between the sham- and verum-stimulated group were found in either of our studies. One reason might be that the temporal relationship between stimulation and clinical impact is not necessarily a linear one, so a delayed onset of action should not yet be excluded. This is even more the case when considering that for major depression a delayed onset effect of rTMS has been reported before in a meta-analysis (Schutter, 2009) and further that the hypothesised mode of action for long-term iTBS effects are changes within the neurotransmitter system as already discussed during the last section. However, up to date, studies that have investigated the long-term effects of rTMS and more specifically iTBS on anxiety symptoms are still missing. On a related account, very recently Reznikov, Binko, Nobrega, & Hamani (2016) investigated the application of deep brain stimulation in areas including the amygdala, the ventral striatum, the hippocampus and the PFC in animal models of post traumatic stress disorder in a review article and found improved fear extinction and accordingly fewer anxiety symptoms. Even though this is not directly comparable to the repeated use of rTMS in humans, the results are encouraging regarding future research in the field of long-term application of brain stimulation methods as clinical treatment options. Equivalently to the inconsistent neurobiological changes, another explanation for the missing stimulation effect in both of our studies might simply be given by a ceiling effect of psychotherapy. Indeed, our therapeutic intervention in study 1 was rather effective as already after the first three sessions a significant reduction in clinical symptom severity could be shown independently of the stimulation group. Moreover,

all patients received an individual therapist-guided exposure session which has been shown to be one of the major mechanisms of action in the treatment of panic disorder/agoraphobia (see section 1.4.1). In line with this assumption, a study by Prasser et al. (2015) where the authors investigated the effect of prefrontal rTMS on depressive symptoms as an add-on to psychotherapy could not find any advantages of verum compared to sham stimulation. Surely, this still does not explain why we could not find any difference in anxiety symptoms during the VR challenge in study 2 where we explicitly refrained from all kinds of additional therapeutic intervention and solely focused on the effects of iTBS on fear-induction. Yet, as presumed above, the mere exposure to the phobic objects might have already induced some kind of habituation effect which is in fact supported by the adaption of our physiological measures in terms of HR and EDA as well as subjectively perceived anxiety. Indeed, even though we did not find an anxiolytic effect of iTBS, on the upside one might say our study underlines the potential of VR exposure in psychotherapy. Even though this dissertation did not focus on the application of VR, it shall be mentioned that it also presents an important field for future studies as it undoubtedly also comprises further valuable possibilities regarding the implementation of exposure therapy considering its capability of simulating situations which would otherwise be hard to realise. Examples might be the exposure to trauma-associated stimuli in patients suffering from PTSD or the confrontation with illegal substances in drug dependence. A clear advantage in this regard is the safety as well as controllability of the virtual situation which may also improve the commitment of some patients who might have otherwise avoided the confrontation with their feared stimuli (Garcia-Palacios, Botella, Hoffman, & Fabregat, 2007; Mitrousia & Giotakos, 2016).

But to get back to the missing iTBS effect in study 2 another reason might simply be that we should have chosen another stimulation site rather than the DLPFC. In this regard, a very recently published study by Herrmann et al. (2016) found an improvement of therapy response in patients suffering from acrophobia after VR exposure when combining it with activating rTMS over the MPFC in the verum- compared to the sham-stimulated group. In more detail, the authors showed a greater reduction of subjectively reported phobic symptoms right after rTMS-augmented VR exposure therapy but not at follow-up three months later. From this,

they concluded that activating rTMS over the MPFC directly influences extinction learning processes per se but not the recall of extinction. However, this finding might still be of therapeutical relevance as it suggests that rTMS may accelerate therapy response which in turn may improve the patients` commitment at the beginning of exposure therapy and thus raises hope in patients who might have otherwise given up when not being able to experience a sense of achievement at the start of therapy. Furthermore, the authors emphasise the importance of the timing of rTMS stimulation as animal studies (Milad, Vidal-Gonzalez, & Quirk, 2004) have shown that infralimbic stimulation only modulates extinction learning when applied during the training situation but not before. A possible explanation for this finding could be an increase in functional connectivity during stimulation. A hint for this assumption is for example given by Kroczek et al. (2016) who found that anodal tDCS during smoking cue exposure led to increased functional connectivity between prefrontal areas responsible for value reinforcement and cognitive control. However, similar to our study the authors could not find any differences in subjectively perceived symptom reduction.

All in all, these studies suggest that the effects of neurostimulation and its associated processes are very sensitive to a number of factors like the timing or the site of stimulation. Moreover, they always have to be regarded in interaction with the prior brain state as well as the stimulation environment, including further therapeutical interventions the stimulated individual may receive. From this point of view, it is not enough to just choose the brain region that should be inhibited or enhanced in order to achieve an improvement of clinical symptoms when designing a study. On the contrary, even when investigating the combination of a brain stimulation technique and a well-controlled exposure situation a number of details need to be considered in order to potentially gain a real add-on effect. To take this reasoning a bit further, it might be necessary to even individually decide which patient profits from what kind of supportive neurostimulation protocol. Taking panic disorder as an example, some patients might mainly suffer from loss of cognitive control as soon as they are confronted with a fear-inducing stimulus, be it an internal one like sensing their own heart beat or an external one like being in a situation similar to one where a panic attack has occurred before. This group of patients may be so overwhelmed by their

feelings of anxiety that even though they are familiar with "the vicious circle of anxiety" on a cognitive level, including the knowledge of their individual triggers for panic attacks, especially at the beginning of psychotherapy, they cannot transpose this knowledge to an adequate plan of action. Consequently, in this group of patients it might make sense to apply, for instance, prefrontal activating rTMS and as add-on intervention in order to enhance cognitive control. However, there might also be a second group of patients that already executes "too much" control by using cognitive avoidance strategies thereby distracting themselves from any kind of fear-inducing sensations or thoughts. In this group of patients, it might on the contrary be more helpful to receive an rTMS treatment which helps them to give up cognitive control and expose themselves to their feeling of anxiety. Taken together, a further explanation for the missing clinical iTBS effect might be that there were different kinds of patients within our treatment group who nevertheless all received the same stimulation independent of whether they tended to use cognitive avoidance strategies or be overwhelmed by their fear during the confrontation with panic-associated stimuli. On a similar account, regarding one-time stimulation prior to a specific situation, a recent study (Möbius et al., 2017) found that negative mood swings in response to sad movie clips were significantly more pronounced after active rTMS over the left DLPFC then after sham stimulation. The authors discuss their findings at odds with the current literature as they used an activating protocol and thus expected less negative mood swings in the active group, which should indeed be expected if the subjects tried to avoid negative feelings. However, considering that only healthy subjects without any affective disorders were included who were instructed to pay attention to the video clips, at least from a "cognitive point of view", it makes sense that increased prefrontal activation may lead to an increased capacity to pay attention to the presented stimuli. And thus, as they were negative ones consequently also produced a more pronounced negative mood induction. Likewise, Shahbabaie et al. (2014) found significant changes in craving intensity to addiction-related stimuli during anodal tDCS in combination with a cue-induced craving task. Interestingly, at rest, meaning when no cues were presented, craving was reduced during active as compared the sham tDCS. However, during cue presentation this pattern reversed and active stimulation induced a significant

increase in craving which the authors interpreted in terms of tDSC-induced saliency of the presented stimuli. Thus, this study also underlines that the effects of neurostimulation are very sensitive to the specific setting and the results depend very much not just on the environment but also the internal cognitive state of the stimulated individual.

In order to complete this dissertation, it can be concluded that in line with the current literature, we could show functional prefrontal alterations within the fear network in different anxiety disorders. These neuronal activation patterns could partly be influenced and normalised by means of iTBS which may be seen as an indicator for its potential as an add-on tool in psychotherapy. However, so far this potential has not yet been sufficiently exploited as can also be seen in the lack of clinical effects in our studies.

In this regard, a major limitation of both our studies was probably that we did not take the state-dependency of iTBS into account. Further, individual factors, like possible biomarkes, which might predict the effectiveness of iTBS were not considered. Aside from that, we did not clearly define the temporal and contextual factors of iTBS stimulation. For example, in study 1 all participants received 15 sessions of iTBS during the first three weeks of the psychoeducational phase of psychotherapy. However, we did not specify whether the stimulation took place before or after the therapeutical sessions as long as the individually chosen time of the day was held constant over all 15 sessions. However, it might make a crucial difference at what time in relation to the specific element of psychotherapy iTBS as an add-on is delivered. In the same matter, it might have also been interesting to vary the timeframe of iTBS application and the phase of psychotherapy, for instance not just applying it during psychoeducation but also during the session when exposure training was taking place. At last, it cannot be ruled out that especially in study 1 where the blinding of our participants was not sufficiently successful a placebo effect might have also influenced our results. In fact, even though in study 2 the blinding was effective, the mere stimulation situation might have induced some kind of placebo effect.

In this regard, in future studies it might be of advantage to include an additional control condition without any iTBS application in order to be able to clearly ascribe

possible iTBS effects to the actual stimulation protocol. Even more importantly though, a lot of further research is needed to clarify under which circumstances what kind of stimulation should be applied in order to receive the desired effects. When not taking such contextual and individual factors into account, it will probably be hard to achieve a consistent clinical add-on effect in terms of symptom reduction in the patients. Thus, future research needs to specifically evaluate these contextual as well as individual factors, including the prior brain state of the stimulated patients, their emotion regulation strategies or the time of iTBS application in order to achieve a supportive therapeutic effect of iTBS or more universally speaking, of different neurostimulation methods in general.

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6. Contribution of the author

For both studies, the author was essentially involved in the development of the research questions. Regarding studiy 1 and the emering manuscripts, the author was further in charge of the collection, analyses as well as interpretation of the fNIRS data. Psychotherapy, the interpretation of the clinical data as well as the completion of the manuscripts was accomplished in collaboration with the second first author who was also responsible for the analysis of the clinical data.

Regarding studiy 2, the author was again responsible for the collection, analyses and interpretation of the fNIRS data as well as the related valence and arousal ratings whereas the second first author mainly analysed the physophysiological data as well as the questionnaire scores. Again, the interpretation of the clinical data and the completion of the manuscripts as well as the collection of the psychophysiological data and VR application were done collectively.

The coauthors supported and contributed at single processing stages, such as study preparation, iTBS application or adaption of the employed paradigms.