

# The effect of bicephalic stimulation of the dorsolateral prefrontal cortex on the attentional bias for threat: A transcranial direct current stimulation study

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**Abstract** Previous stimulation studies demonstrated that the dorsolateral prefrontal cortex (DLPFC) is involved in threat processing. According to a model of emotional processing, an unbalance between the two DLPFCs, with a hyperactivation of right frontal areas, is involved in the processing of negative emotions and genesis of anxiety. In the present study, we investigated the role of the right and left DLPFC in threat processing in healthy women who also completed the State-Trait Anxiety Inventory (STAI). We simultaneously modulated the activity of the right and left dorsolateral prefrontal cortex by applying bicephalic transcranial direct current stimulation (tDCS) before participants completed a modified version of the classic Posner task using threatening and nonthreatening stimuli as spatial cues. Anodal stimulation on the right DLPFC with a simultaneous cathodal stimulation over the left side induced a disengagement bias in individuals with low STAI scores and a facilitation bias in individuals with high STAI scores. Anodal stimulation on the left DLPFC with the simultaneous cathodal stimulation over the right side did not affect threat processing. The findings of the present study provided specific support to the hypothesis that unbalanced activation between left and right hemispheres with enhanced activation of the right DLPFC is critical in early top-down threat processing in healthy individuals.

**Keywords** TDCS · Attention · Threat · Anxiety · DLPFC · Attentional bias

The prefrontal cortex is considered a key structure for processing and responding to positive and negative emotion-related information. According to the well-known valence-asymmetry hypothesis (Davidson & Irwin, 1999), the left and right prefrontal cortex would play different roles in emotion processing, with the right hemisphere preferentially involved in negative emotion processing and the left hemisphere engaged in positive emotion processing.

Different patterns of brain activity in the right and the left hemisphere might also contribute to development of anxiety and depression. For instance, Heller, Nitschke, Etienne, and Miller (1997) proposed that anxious arousal is associated with greater activity in right-hemisphere than in left-hemisphere regions, whereas anxious apprehension is associated with greater left-hemisphere activity (see also Engels et al., 2007; Sass et al., 2010). Moreover, Davidson (1998) proposed a differential involvement of the right and the left prefrontal cortex in anxiety and depression, as a decreased activation in left prefrontal cortex would be related to depression, whereas an increased activation of the right prefrontal cortex would be specific for anxiety.

Several studies demonstrated that healthy people with high anxiety levels, measured with the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), show lower activation of the left dorsolateral prefrontal cortex (DLPFC) and stronger attention allocation on threatening stimuli with respect to healthy people with low anxiety levels (Bishop, Duncan, Brett, & Lawrence, 2004; Bishop, 2007). The possible relationships between anxiety and prefrontal control mechanisms responsible for attention allocation have been specifically addressed in Bishop's

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(2008) model. In this model, trait and state anxiety have been proposed to differently modulate brain activity during threat processing: high trait anxiety would be associated with reduced activation of prefrontal control mechanisms while high state anxiety would be associated with an increase of amygdala activation. According to this model, the failure of the top-down attentional control is considered to be involved in etiology of anxiety and to be responsible for attentional biases for threat (ABTs). However, it should be taken into account that in the abovementioned previous studies, anxiety has been assessed with the STAI, which is also highly correlated with measures of depression (Nitschke, Heller, Imig, McDonald, & Miller, 2001).

ABTs may manifest as an attentive facilitation in detecting threatening compared to other stimuli (facilitation bias), a difficulty in disengaging attention from threatening stimuli (disengagement bias) or an attentional avoidance (avoidance bias) of threats (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Cisler & Koster, 2010). Previous behavioral studies demonstrated that the kind of ABTs shown by healthy individuals is related to stimulus presentation times (PTs). In particular, facilitation bias is usually found when threatening stimuli are presented for 100 to 200 ms (Koster, Crombez, Verschuere, Van Damme, & Wiersema, 2006; Koster, Crombez, Verschuere, Vanvolsem, & De Houwer, 2007; Massar, Mol, Kenemans, & Baas, 2011; Sagliano, Trojano, Amoriello, Migliozzi, & D'Olimpio, 2014). Difficulty in disengagement can be observed at PTs ranging between 100 and 500 ms (Fox, Russo, Bowles, & Dutton, 2001; Koster, Crombez, Verschuere, & De Houwer, 2004; Koster et al., 2006; Sagliano et al., 2014). Attentional avoidance can be found at PTs ranging from 200 ms to seconds (e.g., Koster et al., 2006; Sagliano et al., 2014).

Data from recent stimulation studies evaluating the role of prefrontal cortex and related areas (anterior cingulate cortex and orbitofrontal cortex) in ABTs (e.g., De Raedt et al., 2010) indirectly supported Bishop's model. Vanderhasselt, Baeken, Hendricks, and De Raedt (2011) demonstrated that high-frequency (excitatory) repetitive transcranial magnetic stimulation (HF-rTMS) over the right DLPFC triggered ABT and that this effect was maximum in participants with the highest state anxiety scores. Leyman, De Raedt, Vanderhasselt, and Baeken (2009) reported that a single session of HF-rTMS over the right DLPFC increased attentional biases for threatening stimuli (angry faces), whereas HF-rTMS over the left DLPFC did not lead to significant changes in attentional control. Using an online single-pulse TMS protocol, Sagliano, D'Olimpio, Panico, Gagliardi, and Trojano (2016) found that inhibitory stimulation of the left DLPFC, with the pulse delivered 100 ms (but not 200 ms) after stimulus onset, determined a disengagement bias in high anxious individuals, while the same stimulation determined an attentional avoidance in low anxious individuals. Therefore, this study demonstrated that

the left DLPFC is involved in early top-down threat processing, and that its role is related to individuals' trait anxiety level (assessed by STAI). In a combined HF-rTMS and fMRI study, De Raedt et al. (2010) demonstrated the rTMS over the right DLPFC reduced disengagement bias for threat (angry faces) in healthy women, and decreased activation within the right DLPFC, dorsal anterior cingulate cortex, and left superior parietal gyrus, with increased activity within the right amygdala. Conversely, the stimulation over the left DLPFC determined a reduction of disengagement bias for threat and was associated with increased activation of the right DLPFC, right superior parietal gyrus, dorsal anterior cingulate cortex, and the left orbitofrontal cortex. This study demonstrated that rTMS over the right and the left DLPFC have opposite effects on ABTs: stimulating the right DLPFC might enhance attentional allocation on threatening stimuli, whereas stimulating the left DLPFC might reduce ABTs.

The aim of the present study was to specifically investigate whether ABTs are related to an unbalance in prefrontal activity. Although previous studies emphasized the role of DLPFC in ABTs and in the genesis of anxiety and depression, the possible role of a right–left unbalance has not been specifically investigated. To clarify this issue, we took advantage from the fact that, using anodal and cathodal stimulation, transcranial direct current stimulation (tDCS) can modulate cortical activity with opposite effects on the left and right DLPFC. Indeed, through polarization shifts on the resting membrane potentials in cortical layers (Nitsche et al., 2008), anodal tDCS generally facilitates cortical activity, whereas cathodal tDCS has opposite effects. Indeed, three very recent studies demonstrated that anodal tDCS over the left DLPFC (Heeren et al., 2017), alone or in combination with the attention bias modification procedure (Clarke, Browning, Hammond, Notebaert, & MacLeod, 2014; Heeren, Baeken, Vanderhasselt, Philippot, & De Raedt, 2015), is effective in reducing attention allocation on threat, but these studies did not evaluate the effect of right DLPFC stimulation.

For the purposes of the present study, we employed off-line tDCS with bicephalic montage on healthy participants who were required to perform a modified version of the classic Posner task (so-called exogenous cueing task) using threatening and nonthreatening stimuli as peripheral spatial cues to orient participants' covert attention (Sagliano, D'Olimpio, Tagliatela Scafati, & Trojano, 2015; Weierich, Treat, & Hollingworth, 2008). Since the kind of ABTs elicited in such a task is strongly related to PTs, as briefly recalled above, we adopted three presentation times (100, 200, and 500 ms) to obtain a full range exploration of the effect of tDCS over DLPFC. Moreover, we also assessed whether the effect of tDCS on ABTs is modulated by baseline level of STAI-trait score, as in

previous behavioral (e.g., Fox, 2002; Sagliano et al., 2014) and TMS studies on ABT (Sagliano et al., 2016).

Based on the previous studies reviewed above, we expected that inhibition of the right DLPFC and concurrent activation of the left DLPFC led to a reduced attention allocation or an avoidance of threat. Moreover, the inhibition of the left DLPFC and the concurrent activation of the right DLPFC should lead to an increase of attention allocation (disengagement and facilitation biases) on threat. We also expected that the effect of tDCS stimulation would be modulated by STAI scores.

## Method

### Participants

For recruiting a homogeneous sample and avoiding gender-related differences in brain activation during emotional-related tasks, only female participants were enrolled, consistent with previous studies (e.g., Vanderhasselt et al., 2011). Further selection criteria were right-handedness; lack of any current or past psychiatric, cardiovascular, or neurological disorder; being medication free.

Forty healthy right-handed female participants, aged 18 to 31 years ( $M = 22.95$ ,  $SE = .48$ ), met selection criteria and gave their written informed consent to participate, after having received a complete description of the study.

The entire procedure was approved by the institutional ethics committee of the Department of Psychology, University of Campania “Luigi Vanvitelli,” and was conducted in accordance with the ethical standards of the Helsinki declaration.

### State-Trait Anxiety Inventory

All participants completed the State-Trait Anxiety Inventory (Spielberger et al., 1983), consisting of two 20-item scales. The STAI-State subscale requires respondents to rate how they feel “right now...at this moment” using a 4-point scale (1 = *not at all*, 4 = *very much so*) in response to a series of self-descriptive statements. The STAI-Trait subscale asks respondents to rate how they “generally” feel using a 4-point scale (1 = *almost never*, 4 = *almost always*) in response to a series of self-descriptive statements. These subscales have been demonstrated to have solid psychometric properties.

### Exogenous cueing task

All participants performed an exogenous spatial cueing task similar to those used in previous studies (Sagliano et al., 2015; Sagliano et al., 2014) with threatening ( $n = 20$ ) and nonthreatening ( $n = 20$ ) cues selected from the International Affective

Picture System (Lang, Bradley, & Cuthbert, 2008). As in previous studies, we selected familiar scenes of animals, people, or natural events on the basis of a previous assessment of their valence, arousal, and threatening ratings. To obtain such ratings, we required 30 undergraduate students (not taking part in the present experiment) to judge images in terms of valence and arousal, and to rate how threatening each image was on a scale from zero to 4. Threatening stimuli (e.g., a spider, a group of armed boys, a gun) were selected among those with negative valence ( $<4.5$ ) and high arousal ( $>5.5$ ) scores, whereas selected nonthreatening stimuli (e.g., a wood, a man at window, a cup) had intermediate valence and arousal scores ( $>4.5$  and  $<5.5$ ). Selected threatening images were assessed as the most threatening (mean score of threat degree = 2.8; range: 2.5–4.0), whereas the nonthreatening images were considered as those least threatening (mean score of threat degree = 0.65; range: 0–1;  $p < .001$ ).

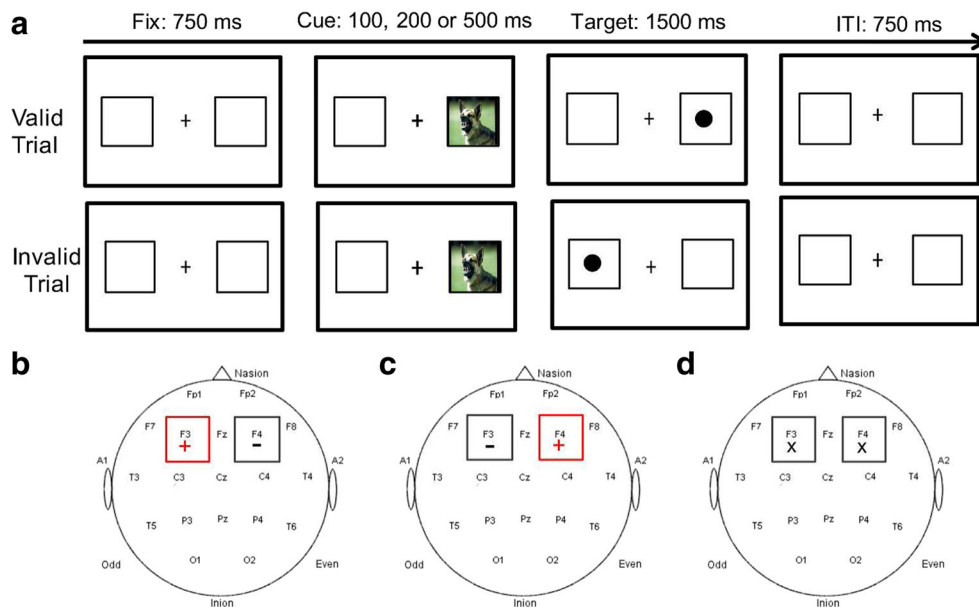
Each trial consisted in a fixation cross (+) flanked by two blank squares ( $340 \times 340$  pixels) on its right and left side (300 pixel from the fixation cross; visual angle:  $11.30^\circ$  at a viewing distance of 50 cm) presented for 750 ms and followed by a cue (a threatening or nonthreatening image;  $300 \times 300$  pixels) that randomly appeared for one of three presentation times (100, 200, or 500 ms) in one of the two squares. Each image appeared twice in the right square and twice in the left square for each presentation time. Each stimulus was presented six times, for a total number of 240 trials.

After cue presentation, a dot (1 cm diameter) appeared in one of the two squares, in the same (valid trial) or in the opposite (invalid trial) position as the cue for 1,500 ms or until participants’ response (see Fig. 1). Intertrial interval was 750 ms. Valid trials ( $n = 192$ , 80%; 96 threatening and 96 nonthreatening) and invalid trials ( $n = 48$ , 20%; 24 threatening and 24 nonthreatening) were presented in a randomized order for a total of 240 trials.

The participants, comfortably seated in a quiet room, were required to keep their eyes on the fixation cross and to respond, as fast and accurately as possible, pressing a right key (*l*) on the keyboard when the target appeared on the right and a left key (*a*) when the target appeared on the left. For each trial, both accuracy and response times (RTs) were recorded.

### Transcranial direct current stimulation

Stimulation has been delivered by a battery-driven, constant current stimulator (BrainSTIM, EMS Medical, Italy) using a pair of surface saline-soaked  $5 \times 5$  cm sponge electrodes (area =  $25 \text{ cm}^2$ ; pads and electrodes were roughly of the same size). For left anodal/right cathodal stimulation (see Fig. 1) the anodal electrode was placed over the left DLPFC (F3), while the cathodal electrode was placed over the right DLPFC (F4) based on the international 10-20 system (Klem, Luders, Jasper, & Elger, 1999). For F4 anodal/F3 cathodal stimulation,



**Fig. 1** Examples of threatening valid (*top row*) and invalid trials (*bottom row*) of the exogenous cueing task (**a**). Schematic overview of the stimulation protocol during the F3 anodal/F4 cathodal (**b**), the F4 anodal/F3 cathodal (**c**) and the sham condition (**d**). (Color figure online)

polarity of electrodes was inverted with the anodal electrode placed over the right DLPFC and the cathodal electrode placed over the left DLPFC (see Fig. 1). Within each stimulation session, a constant current of 1 mA (current density = .04 mA/cm<sup>2</sup>) was applied for 15 min, with a linear fade in/fade out of 20 s.

For sham stimulation, duration of session, electrode position and fade-in/fade-out time were the same as in real tDCS stimulation; however, the current was ramped down after 30 seconds. This procedure ensured that participants felt the same itching sensation at the beginning of tDCS as when they were undergoing the active stimulation conditions (Gandiga et al., 2006).

## Procedure

The study consisted of three sessions. In the first session, participants completed the STAI and performed the modified exogenous cueing task as a pretest training (pre-tDCS assessment). At the end of the task, each participant underwent one of the tDCS sessions: real F3 anodal/F4 cathodal stimulation, real F4 anodal/F3 cathodal stimulation, sham tDCS. After the stimulation, participants repeated the modified exogenous cueing task to evaluate the effect of tDCS on attentional biases (post-tDCS assessment).

In the second and third sessions, participants first completed the STAI (only State subscale) and then performed one of the remaining stimulation sessions followed by the task.

Each participant performed three separate tDCS sessions: real F3 anodal/F4 cathodal stimulation, real F4 anodal/F3 cathodal stimulation, and Sham tDCS at least 1 week apart

to avoid carryover effects. The session order was counterbalanced across participants.

## Results

Data from one participant were excluded from the analysis due to technical problems with RTs recording.

### Group characteristics

Mean trait anxiety score for the sample was 44.23 ( $SE = 1.49$ ). State anxiety scores in the three stimulations sessions did not differ in the three conditions as shown by three paired  $t$  tests (F3 anodal/F4 cathodal:  $M = 44.23$ ,  $SE = 1.49$ ; F4 anodal/F3 cathodal:  $M = 37.64$ ,  $SE = 1.52$ ; sham:  $M = 35.85$ ,  $SE = 1.57$ ;  $p > .05$ , for all the comparisons).

### Exogenous cueing task: omnibus analysis on RTs

Data were cleaned removing errors trials (1% of the total). A first omnibus ANCOVA  $4 \times 2 \times 2 \times 3$  with four within-subjects factors (stimulation: pre-tDCS, F3 anodal/F4 cathodal, F4 anodal/F3 cathodal, sham; valence: threatening, non-threatening; validity: valid, invalid; PT: 100 ms, 200 ms, 500 ms) and the STAI-Trait score as a covariate was conducted on RTs. Stimulation and valence has been considered variables of interest for planned comparisons. For this and all subsequent analyses, significant effects were followed up with Bonferroni-corrected post hoc comparisons, in which the computed alpha values are multiplied by the number of post



hoc tests to obtain corrected *p* values and control familywise error rate.

This analysis revealed the following significant main effects: stimulation condition,  $F(3, 111) = 2.83, p = .04, \eta_p^2 = .07$ , with longer RTs for pre-tDCS condition ( $M = 366.84, SE = 7.54$ ) compared to the other three conditions (F3 anodal/F4 cathodal:  $M = 341.35, SE = 5.88$ ; F4 anodal/F3 cathodal;  $M = 338.45, SE = 5.67$ ; sham:  $M = 341.84, SE = 6.7$ ); validity,  $F(1, 37) = 7.93, p < .01, \eta_p^2 = .18$ , with slower RTs for invalid ( $M = 368.56, SE = 7.22$ ) than for valid trials ( $M = 325.68, SE = 5.72$ ); presentation time,  $F(2, 74) = 4.10, p = .02, \eta_p^2 = .10$ , with faster responses for longer PTs (100 ms = 361.41; 200 ms = 344.14; 500 ms = 335.8; all different from each other at  $p < .01$ ). Moreover, this analysis revealed the following significant interactions: Stimulation  $\times$  Validity,  $F(3, 111) = 4.43, p < .01, \eta_p^2 = .11$ ; Stimulation  $\times$  Valence  $\times$  PT,  $F(6, 37) = 3.92, p < .001, \eta_p^2 = .62$ ; and Stimulation  $\times$  Validity  $\times$  Valence,  $F(3, 111) = 3.51, p = .02, \eta_p^2 = .09$ . The last third order interaction remained significant also considering STAI-Trait scores as a covariate,  $F(3, 111) = 3.49, p = .02, \eta_p^2 = .08$ .

Regarding to the Stimulation  $\times$  Validity  $\times$  Valence interaction, individuals were slower in responding to both threatening and nonthreatening stimuli for both valid and invalid trials in pre-tDCS condition compared to all the other conditions ( $p < .01$  for all the comparisons) and were faster in responding to neutral valid trial after F4 anodal/F3 cathodal condition than in sham condition. Moreover, individuals were faster to respond to threatening valid trials compared to nonthreatening valid trials only after sham stimulation (see Table 1).

In synthesis, the above omnibus analysis demonstrated that individuals were significantly slower in pre-tDCS condition, and that this condition markedly differed from all the others. For this reason, we performed separate ANCOVAs for pre-tDCS and post-tDCS conditions.

A  $2 \times 2 \times 3$  ANCOVA on correct RTs of the pre-tDCS task with three within-subjects factors (valence: threatening, non-threatening; validity: valid, invalid; PT: 100 ms, 200 ms, 500 ms) and STAI-Trait score only revealed a marginally significant Valence  $\times$  PT interaction,  $F(2, 74) = 3.15, p = .049, \eta_p^2 = .08$ , as individuals were slower to respond to threatening stimuli presented for 100 ms ( $M = 388.59, SE = 9.30$ ) compared to nonthreatening ( $M = 382.99, SE = 8.72$ ). No other significant main effect or interaction were significant.

### Analysis of RTs for post-tDCS tasks

A  $3 \times 2 \times 2 \times 3$  ANCOVA, with four within-subjects factors (stimulation: F3 anodal/F4 cathodal, F4 anodal/F3 cathodal, sham; valence: threatening, nonthreatening; validity: valid, invalid; PT: 100 ms, 200 ms, 500 ms) and STAI-Trait score as a covariate was run on correct RTs (see Table 2). This analysis showed a significant effect of validity,  $F(1, 37) = 9.29, p = .004, \eta_p^2 = .20$ , with slower RTs for invalid ( $M =$

**Table 1** Mean (and standard error, *SE*) RTs in the exogenous cueing task as a function of validity (invalid, valid), valence (threat, nonthreat), and stimulation condition

		Threat		Nonthreat	
		Mean	SE	Mean	SE
Invalid	Pre-tDCS	385.96	8.72	384.78	8.72
	F3-A/F4-C	364.93	7.75	360.93	7.56
	F4-A/F3-C	363.53	7.36	359.84	7.47
	Sham	366.41	8.62	362.12	8.42
Valid	Pre-tDCS	348.61	7.92	348.03	7.34
	F3-A/F4-C	319.13	6.14	320.42	5.76
	F4-A/F3-C	316.50	5.72	313.94	5.66
	Sham	317.12	6.48	321.72	6.55

Note. Pre-tDCS, F3-A/F4-C = F3 anodal/F4 cathodal session; F4-A/F3-C = F4 anodal/F3 cathodal session; Sham = sham session

362.96,  $SE = 7.23$ ) than for valid trials ( $M = 318.14, SE = 5.50$ ). Moreover, we found the following significant interactions: Stimulation  $\times$  Validity  $\times$  Valence,  $F(2, 74) = 4.57, p = .01, \eta_p^2 = .11$ ; Stimulation  $\times$  Validity  $\times$  Valence  $\times$  Trait Anxiety Scores,  $F(2, 74) = 4.49, p = .01, \eta_p^2 = .11$ ; Stimulation  $\times$  Valence  $\times$  PT,  $F(4, 148) = 3.71, p = .007, \eta_p^2 = .09$ ; Stimulation  $\times$  Valence  $\times$  PT  $\times$  Trait Anxiety Scores,  $F(4, 148) = 3.42, p = .01, \eta_p^2 = .08$ ; Stimulation  $\times$  Validity  $\times$  Valence  $\times$  PT,  $F(4, 148) = 2.61, p = .04, \eta_p^2 = .07$ . No other significant main effect or interaction emerged.

Comparisons for the high order interaction revealed that, after F4 anodal/F3 cathodal stimulation individuals were faster in responding to valid trial with threatening stimuli

**Table 2** Mean (and standard error, *SE*) RTs in the exogenous cueing task as a function of validity (invalid, valid), presentation time (PT: 100, 200, 500 ms), valence (threat, nonthreat), and stimulation condition

Validity	PT	Valence	F3-A/F4-C		F4-A/F3-C		Sham	
			Mean	SE	Mean	SE	Mean	SE
Invalid	100	Threat	377.26	8.8	371.48	8.2	375.71	9.96
		Nonthreat	370.5	8.17	365.17	8.21	368.4	10.38
	200	Threat	361.09	7.95	365.93	7.69	361.94	8.21
		Nonthreat	357.54	7.92	357.56	8.54	365.51	10.21
	500	Threat	356.44	8.47	353.19	8.61	361.58	10.07
		Nonthreat	354.74	8.7	356.8	8.79	352.45	7.77
Valid	100	Threat	333.91	6.74	336.08	6.33	335.82	6.96
		Nonthreat	337.31	6.76	330.31	6.09	337.54	7.19
	200	Threat	315.5	6.67	307.24	5.55	311.1	6.36
		Nonthreat	317.87	6.1	311.74	5.6	319.81	6.33
	500	Threat	307.98	6.6	306.17	6.65	304.43	7.45
		Nonthreat	306.09	6.04	299.77	6.43	307.81	7.46

Note. F3-A/F4-C = F3 anodal/F4 cathodal session; F4-A/F3-C = F4 anodal/F3 cathodal session; Sham = sham session

presented for 200 ms ( $M = 307.24$ ,  $SE = 5.54$ ) compared to F3 anodal/F4 cathodal condition ( $M = 315.50$ ,  $SE = 6.67$ ). Moreover, after F3 anodal/F4 cathodal stimulation, individuals were slower to respond to valid trials with threatening stimuli presented for 100 ms ( $M = 336.08$ ,  $SE = 6.33$ ) compared to nonthreatening stimuli ( $M = 330.31$ ,  $SE = 5.55$ ). On the contrary, in the sham condition, individuals were faster to respond to valid trials with threatening stimuli presented for 200 ms ( $M = 311.1$ ,  $SE = 6.36$ ) compared to nonthreatening stimuli ( $M = 319.81$ ,  $SE = 6.33$ ).

Reliability estimates using the split-half method on RTs of all the stimulation conditions revealed that the task did produce acceptable levels of reliability (see Supplementary Material 1).

### Bias scores

After Koster et al. (2006), we computed the following bias scores: engagement score (FB: RTvalid/nonthreatening cue - RTvalid/threatening cue), and disengagement score (DB: RTinvalid/threatening cue - RTinvalid/nonthreatening cue). Positive engagement scores indicate a prompt attentional capture by threatening stimuli compared to nonthreatening stimuli (facilitation bias). Positive values on disengagement score indicate longer attentional holding by threatening stimuli compared to nonthreatening stimuli (disengagement bias). Negative values for both scores indicate a tendency to avoid threatening stimuli (avoidance bias). A value not different from zero at any score means lack of ABTs (i.e., no difference in processing of threatening vs. nonthreatening cues).

In order to search for the presence of differences between the stimulation session and/or the presentation times, a  $3 \times 3$  ANCOVA, with two within-subject factors (stimulation: F3 anodal/F4 cathodal, F4 anodal/F3 cathodal, sham; PT: 100 ms, 200 ms, 500 ms) and the STAI scores as covariate has been separately conducted on engagement and disengagement scores for pre-tDCS and post-tDCS tasks.

The ANCOVA on pre-tDCS task on both engagement and disengagement scores did not show any significant effect or interaction ( $p > .05$ ).

On post-tDCS tasks, ANCOVA on engagement score did not reveal any significant effect or interactions (all  $ps > .05$ ).

The ANCOVA on disengagement scores showed significant Stimulation  $\times$  STAI Scores  $F(2, 74) = 3.22$ ,  $p = .04$ ,  $\eta_p^2 = .08$ ; Stimulation  $\times$  PT,  $F(4, 148) = 5.17$ ,  $p = .001$ ,  $\eta_p^2 = .12$ ; and Stimulation  $\times$  PT  $\times$  STAI Scores interactions,  $F(4, 148) = 4.95$ ,  $p = .001$ ,  $\eta_p^2 = .12$ . Post hoc comparisons did not reveal differences as a function of the stimulation conditions and the presentation times.

To evaluate the presence of ABTs in each condition, single-sample  $t$  tests were computed to determine whether each bias score was significantly different from zero. As previous ANCOVAs performed on both RT and bias scores showed

that STAI scores interacted with the other factors in modulating ABTs, we computed engagement and disengagement scores separately in participants with high ( $n = 20$ ) or low ( $n = 19$ ) STAI-Trait scores, based on a median split (median STAI = 43; mean STAI scores of the two subgroups are reported in Supplementary Material 2). Mean of biases scores for all the conditions are reported in Supplementary Material 3. One-sample  $t$  tests to search for ABTs were then conducted for the two subgroups for both pre-tDCS and post-tDCS tasks. In pre-tDCS, this analysis did not reveal any significant engagement or disengagement bias score. In the post-tDCS, after F4 anodal/F3 cathodal stimulation, the disengagement bias in participants with low STAI scores,  $t = 2.49$ ,  $p = .02$ , and the facilitation bias in participants with high STAI scores,  $t = 3.12$ ,  $p < .01$ , were significantly different from zero when the cue was presented for 200 ms (see Fig. 2).

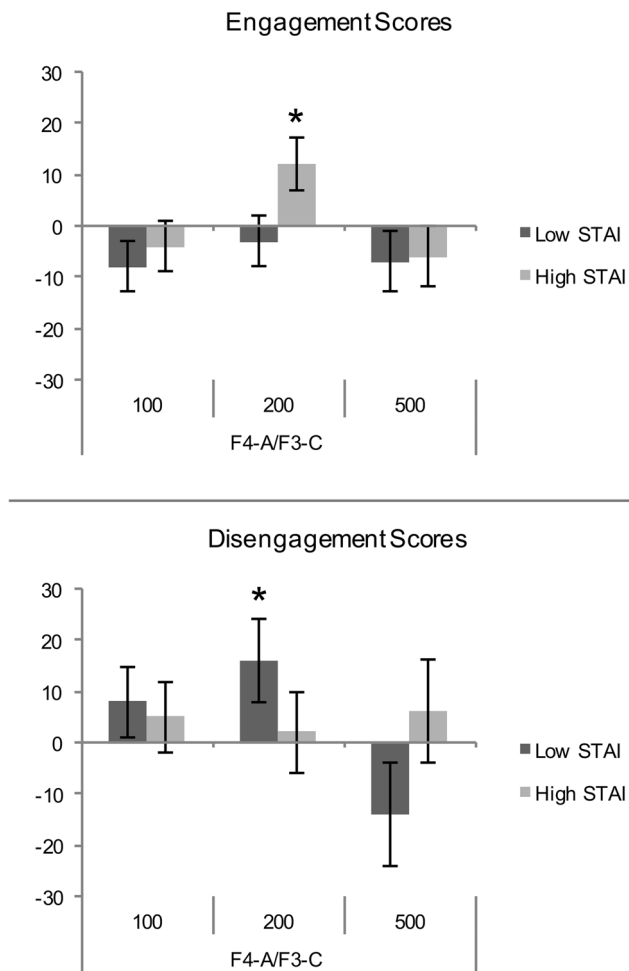
Reliability estimates using the split-half method on bias scores of all the stimulation conditions revealed low levels of reliability (see Supplementary Material 1).

### Discussion

The present study aimed at investigating the effect of an unbalanced activation of right and left DLPFC on ABTs by means of a tDCS protocol, simultaneously modulating the activity of right and left DLPFC. We observed that anodal stimulation over the right DLPFC and simultaneous cathodal stimulation over the left DLPFC determined a disengagement bias in individuals with high STAI scores and a facilitation bias in individuals with low STAI scores when stimuli were presented at 200 ms PT.

The presence of ABTs at 200 ms of stimulus presentation is congruent previous behavioral studies (Amir, Elias, Klumpp, & Przeworski, 2003; Derryberry & Reed, 2002; Fox, 2002; Fox et al., 2001; Sagliano et al., 2015; Sagliano et al., 2014) demonstrating that facilitation and disengagement biases are specifically related to early threat processing. Therefore, our results are compatible with the basic idea that DLPFC is involved in early top-down attentional processing (see also Eysenck, Derakshan, Santos, & Calvo, 2007), as shown in a study combining magnetoencephalography and TMS in which stimulation over the right DLPFC affected early (110170 ms) processing of fearful faces (Zwanzger et al., 2014).

More importantly, our data nicely fitted the hypothesis that ABTs are related to an unbalance between the activity of right and left DLPFC with a predominant role of the right side in attentional allocation on threat, in line with previous neuroimaging and stimulation studies (e.g., De Raedt et al., 2010). A specific role of the right prefrontal cortex in processing negative emotions has been also suggested by Davidson (1998),



**Fig. 2** Mean bias scores (facilitation bias and disengagement bias) of F4 anodal/F3 cathodal condition (F4-A/F3-C) as a function of presentation times (PT: 100, 200, 500 ms) for individuals with low and high STAI scores (*bars* represent standard errors). Mean bias scores for the other stimulation conditions are reported in Supplementary Material 1. Asterisks indicate significant differences between the bias score and zero. \* $p < .05$

who proposed that two systems mediating different forms of motivation and emotion exist: the approach system and the withdrawal system. The approach system, related to the activity of the left prefrontal cortex, would facilitate appetitive behavior and generate positive affects (e.g., enthusiasm and pride). The withdrawal system, related to the activity of the right prefrontal cortex, would prompt withdrawal from aversive stimuli and generate negative affects (e.g., disgust and fear). Moreover, Davidson (1998) suggested that unbalance in prefrontal activity might predict individual differences in emotional reactions, and that a decreased activation in the left prefrontal cortex may be specific for depression, whereas an increased activation of the right prefrontal cortex may be specific for anxiety. In line with the model proposed by Davidson and Irwin (1999), we demonstrated that involvement of DLPFC in early top-down threat processing is dependent on hemispheric functional asymmetry in emotional processing,

as left DLPFC inhibition and right DLPFC activation caused an increase of the attention allocation on threatening stimuli with a facilitation bias in individuals with high STAI scores and a disengagement bias in participants with low STAI score. As previous study (Nitschke et al., 2001) demonstrated that STAI score correlates with other measures of depression, it is possible that our results are related to participants' negative affect rather than being specific for trait anxiety level. In other terms, anodal stimulation over the right hemisphere and cathodal stimulation over the left DLPFC could have increased hemispheric asymmetry responsible for attentional biases in both anxiety and depression.

Our data can also be interpreted in the light of the model proposed by Corbetta and Shulman (2002). In their model, the authors distinguished two segregated brain networks with different attentional functions: the goal-directed (top-down) system, related to activity of the intraparietal cortex and superior frontal cortex, would be responsible for selection for stimuli and responses; the bottom-up system, related to activity of the temporoparietal cortex and inferior frontal cortex and largely lateralized to the right hemisphere, would be specialized for detection of behaviorally relevant stimuli, particularly when they are salient or unexpected. In our study, the anodal stimulation over the right DLPFC and the simultaneous cathodal stimulation over the left DLPFC could have determined an unbalance of the two systems, with decreased recruitment of goal-directed system and increased recruitment of bottom-up processes.

It is important to underline that the present findings are also compatible with a different theoretical perspective, according to which the biases found after right DLPFC anodal and left DLPFC cathodal stimulation might be related to decreased down-regulation from the left DLPFC on the amygdala (De Raedt & Koster, 2010). Cisler and Koster (2010) proposed a cognitive model of attentional bias for threat in anxiety on the basis of previous neuroimaging studies (e.g., Bishop et al., 2004). According to this model, ABTs are determined by a dysfunctional top-down control of the amygdala with an increase of attention for threat. Data from available TMS studies (De Raedt et al., 2010; Vanderhasselt et al., 2011) were in line with this model, as they revealed that a single session of rTMS over the right DLPFC led to a stronger disengagement bias for threat, likely related to higher amygdala activation, while the left DLPFC stimulation decreased disengagement bias (De Raedt et al., 2010). Our data were consistent with the idea that increased attention for threat after DLPFC stimulation might be related to failure in top-down attentional control and, in particular, in reduced down-regulation of the amygdala by the left DLPFC.

In contrast with previous tDCS studies (Clarke et al., 2014; Heeren et al., 2015; Heeren et al., 2017) demonstrating the efficacy of anodal tDCS over the left DLPFC in reducing attention allocation on threat, here we did not find any effects

after F3 anodal/F4 cathodal stimulation. We could suggest that the lack of effect of this stimulation condition might be related to the characteristics of our sample, not showing any ABTs in pre-tDCS task. However, this hypothesis could be investigated by studies assessing the effect of the unbalance in DLPFC activity in individuals showing ABTs before stimulation.

Several characteristics of our study can limit generalization of findings since we only included female participants to ensure homogeneity of the sample and only employed negative stimuli not allowing us to elucidate whether stimulation of the right and left DLPFC also affected positive emotion processing. Moreover, in the present study, we only used STAI as a measure of psychological aspects, potentially modulating the ABTs after tDCS, whereas future studies might consider employing more specific measures of anxiety, depression, and negative affect (see Nitschke et al., 2001).

The choice of an exogenous cueing task similar to those employed in previous stimulation studies (De Raedt et al., 2010; Vanderhasselt et al., 2011) was aimed to compare the present with previous data easily. However, the exogenous cueing task we used has been criticized (Clarke, Macleod, & Guastella, 2013), as it does not allow discriminating components of attention (engagement and disengagement). It would be interesting to directly compare different attentional tasks (such as the dot-probe task and the exogenous cueing task) in within-subject designs in further behavioral and stimulation studies, since study specifically addressing this issue are not yet available. In this respect, it could also be assessed whether similar results can be obtained via a discrimination task instead of a spatial detection task as in several previous studies (e.g., Cisler & Olatunji, 2010; Mogg, Holmes, Garner, & Bradley, 2008).

The low reliability of bias scores, as assessed by the split-half method, might induce some caution in generalizing the present findings, but this finding is in line with previous studies using the dot-probe paradigm (Dear, Sharpe, Nicholas, & Refshauge, 2011; Staugaard, 2009; Waechter, Nelson, Wright, Hyatt, & Oakman, 2014). As suggested by Schmukle (2005), the low reliability could explain the lack of the replicable effects of the attentional task for the evaluation of the attentional biases but does not preclude assessment of bias scores comparing different experimental treatments in nonclinical individuals.

Last, differently from most recent tDCS studies (Clarke et al., 2014; Heeren et al., 2015), we employed an off-line stimulation protocol allowing our participants to perform the task without worries about sensations like tingling or itching under the electrodes. As a previous study (Reinhart & Woodman, 2014) demonstrated that 20-minute tDCS at 1.5 mA intensity over the medial-frontal cortex can affect behavior and brain activity up to 5 hours, we could be confident that our stimulation determined an effect lasting until the

end of our task (for a review, see Reinhart, Cosman, Fukuda, & Woodman, 2017).

Despite these limitations, the present study contributed to the literature on the modulation of ABTs by means of stimulation techniques, as it is the first to investigate the effect of tDCS over the frontal areas with a bicephalic montage on ABTs. In particular, after the recent studies on the efficacy of tDCS over the left DLPFC combined with the attentional bias modification procedure (Clarke et al., 2014; Heeren et al., 2015), our data might pave the way for possible employment of tDCS with bicephalic montage in clinical settings.

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## References

- Amir, N., Elias, J., Klumpp, H., & Przeworski, A. (2003). Attentional bias to threat in social phobia: Facilitated processing of threat or difficulty disengaging attention from threat? *Behaviour Research and Therapy*, *41*(11), 1325–1335. doi:10.1016/S0005-7967(03)00039-1
- Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & van IJzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study. *Psychological Bulletin*, *133*(1), 1–24. doi:10.1037/0033-2909.133.1.1
- Bishop, S. J. (2007). Neurocognitive mechanisms of anxiety: An integrative account. *Trends in Cognitive Sciences*, *11*(7), 307–316. doi:10.1016/j.tics.2007.05.008
- Bishop, S. J. (2008). Neural mechanisms underlying selective attention to threat. *Annals of the New York Academy of Sciences*, *1129*, 141–152. doi:10.1196/annals.1417.016
- Bishop, S., Duncan, J., Brett, M., & Lawrence, A. D. (2004). Prefrontal cortical function and anxiety: Controlling attention to threat-related stimuli. *Nature Neuroscience*, *7*(2), 184–188. doi:10.1038/nn1173
- Cisler, J. M., & Koster, E. H. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. *Clinical Psychology Review*, *30*(2), 203–216. doi:10.1016/j.cpr.2009.11.003
- Cisler, J. M., & Olatunji, B. O. (2010). Components of attentional biases in contamination fear: Evidence for difficulty in disengagement. *Behaviour Research and Therapy*, *48*(1), 74–78. doi:10.1016/j.brat.2009.09.003
- Clarke, P. J., Browning, M., Hammond, G., Notebaert, L., & MacLeod, C. (2014). The causal role of the dorsolateral prefrontal cortex in the modification of attentional bias: Evidence from transcranial direct current stimulation. *Biological Psychiatry*, *76*(12), 946–952. doi:10.1016/j.biopsych.2014.03.003
- Clarke, P. J., Macleod, C., & Guastella, A. J. (2013). Assessing the role of spatial engagement and disengagement of attention in anxiety-linked attentional bias: A critique of current paradigms and suggestions for future research directions. *Anxiety, Stress, and Coping*, *26*(1), 1–19. doi:10.1080/10615806.2011.638054
- Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*, *3*(3), 201–215. doi:10.1038/nrn755
- Davidson, R. J. (1998). Affective style and affective disorders: Perspectives from affective neuroscience. *Cognition and Emotion*, *12*(3), 307–330.



- Davidson, R. J., & Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. *Trends in Cognitive Sciences*, 3(1), 11–21.
- Dear, B. F., Sharpe, L., Nicholas, M. K., & Refshauge, K. (2011). The psychometric properties of the dot-probe paradigm when used in pain-related attentional bias research. *The Journal of Pain*, 12(12), 1247–1254.
- De Raedt, R., & Koster, E. H. (2010). Understanding vulnerability for depression from a cognitive neuroscience perspective: A reappraisal of attentional factors and a new conceptual framework. *Cognitive, Affective, & Behavioral Neuroscience*, 10(1), 50–70. doi:10.3758/CABN.10.1.50
- De Raedt, R., Leyman, L., Baeken, C., Van Schuerbeek, P., Luybaert, R., Vanderhasselt, M. A., & Dannlowski, U. (2010). Neurocognitive effects of HF-rTMS over the dorsolateral prefrontal cortex on the attentional processing of emotional information in healthy women: An event-related fMRI study. *Biological Psychology*, 85(3), 487–495. doi:10.1016/j.biopsycho.2010.09.015
- Derryberry, D., & Reed, M. A. (2002). Anxiety-related attentional biases and their regulation by attentional control. *Journal of Abnormal Psychology*, 111(2), 225–236.
- Engels, A. S., Heller, W., Mohanty, A., Herrington, J. D., Banich, M. T., Webb, A. G., & Miller, G. A. (2007). Specificity of regional brain activity in anxiety types during emotion processing. *Psychophysiology*, 44(3), 352–363. doi:10.1111/j.1469-8986.2007.00518.x
- Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion*, 7(2), 336–353. doi:10.1037/1528-3542.7.2.336
- Fox, E. (2002). Processing emotional facial expressions: The role of anxiety and awareness. *Cognitive, Affective, & Behavioral Neuroscience*, 2(1), 52–63.
- Fox, E., Russo, R., Bowles, R., & Dutton, K. (2001). Do threatening stimuli draw or hold visual attention in subclinical anxiety? *Journal of Experimental Psychology: General*, 130(4), 681–700. doi:10.1037/0096-3445.130.4.681
- Gandiga, P. C., Hummel, F. C., & Cohen, L. G. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clinical neurophysiology*, 117(4), 845–850.
- Heeren, A., Baeken, C., Vanderhasselt, M. A., Philippot, P., & De Raedt, R. (2015). Impact of anodal and cathodal transcranial direct current stimulation over the left dorsolateral prefrontal cortex during attention bias modification: An eye-tracking study. *PLoS ONE*, 10(4), e0124182. doi:10.1371/journal.pone.0124182
- Heeren, A., Billieux, J., Philippot, P., De Raedt, R., Baeken, C., de Timary, P., ... Vanderhasselt, M. A. (2017). Impact of transcranial direct current stimulation on attentional bias for threat: A proof-of-concept study among individuals with social anxiety disorder. *Social Cognitive and Affective Neuroscience*, 12(2), 251–260. doi:10.1093/scan/nsw119
- Heller, W., Nitschke, J. B., Etienne, M. A., & Miller, G. A. (1997). Patterns of regional brain activity differentiate types of anxiety. *Journal of Abnormal Psychology*, 106(3), 376–385.
- Klem, G. H., Luders, H. O., Jasper, H. H., & Elger, C. (1999). The twenty electrode system of the International Federation. *Electroencephalography and Clinical Neurophysiology*, 52(Suppl.), 3–6.
- Koster, E. H., Crombez, G., Verschuere, B., Van Damme, S., & Wiersma, J. R. (2006). Components of attentional bias to threat in high trait anxiety: Facilitated engagement, impaired disengagement, and attentional avoidance. *Behaviour Research and Therapy*, 44(12), 1757–1771. doi:10.1016/j.brat.2005.12.011
- Koster, E. H., Crombez, G., Verschuere, B., Vanvolsem, P., & De Houwer, J. (2007). A time-course analysis of attentional cueing by threatening scenes. *Experimental Psychology*, 54(2), 161–171. doi:10.1027/1618-3169.54.2.161
- Koster, E. H., Crombez, G., Verschuere, B., & De Houwer, J. (2004). Selective attention to threat in the dot probe paradigm: Differentiating vigilance and difficulty to disengage. *Behaviour Research and Therapy*, 42(10), 1183–1192. doi:10.1016/j.brat.2003.08.001
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual* (Technical Report A-8). Gainesville: University of Florida.
- Leyman, L., De Raedt, R., Vanderhasselt, M.-A., & Baeken, C. (2009). Influence of high-frequency repetitive transcranial magnetic stimulation over the dorsolateral prefrontal cortex on the inhibition of emotional information in healthy volunteers. *Psychological Medicine*, 39, 1019–1028. doi:10.1017/S0033291708004431
- Massar, S. A., Mol, N. M., Kenemans, J. L., & Baas, J. M. (2011). Attentional bias in high- and low-anxious individuals: Evidence for threat-induced effects on engagement and disengagement. *Cognition & Emotion*, 25(5), 805–817. doi:10.1080/02699931.2010.515065
- Mogg, K., Holmes, A., Garner, M., & Bradley, B. P. (2008). Effects of threat cues on attentional shifting, disengagement and response slowing in anxious individuals. *Behaviour Research and Therapy*, 46, 656–667. doi:10.1016/j.brat.2008.02.011
- Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., ... Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*, 1(3), 206–223. doi:10.1016/j.brs.2008.06.004
- Nitschke, J. B., Heller, W., Imig, J. C., McDonald, R. P., & Miller, G. A. (2001). Distinguishing dimensions of anxiety and depression. *Cognitive Therapy and Research*, 25(1), 1–22.
- Reinhart, R. M., Cosman, J. D., Fukuda, K., & Woodman, G. F. (2017). Using transcranial direct-current stimulation (tDCS) to understand cognitive processing. *Attention, Perception, & Psychophysics*, 79(1), 3–23. doi:10.3758/s13414-016-1224-2
- Reinhart, R. M., & Woodman, G. F. (2014). Causal control of medial-frontal cortex governs electrophysiological and behavioral indices of performance monitoring and learning. *Journal of Neuroscience*, 34(12), 4214–4227. doi:10.1523/JNEUROSCI.5421-13.2014
- Sagliano, L., D'Olimpio, F., Panico, F., Gagliardi, S., & Trojano, L. (2016). The role of the dorsolateral prefrontal cortex in early threat processing: A TMS study. *Social Cognitive and Affective Neuroscience*. doi:10.1093/scan/nsw105
- Sagliano, L., D'Olimpio, F., Tagliatela Scafati, I., & Trojano, L. (2015). Eye movements reveal mechanisms underlying attentional biases towards threat. *Cognition & Emotion*, 1–8. doi:10.1080/02699931.2015.1055712
- Sagliano, L., Trojano, L., Amoriello, K., Migliozzi, M., & D'Olimpio, F. (2014). Attentional biases toward threat: The concomitant presence of difficulty of disengagement and attentional avoidance in low trait anxious individuals. *Frontiers in Psychology*, 5, 685. doi:10.3389/fpsyg.2014.00685
- Sass, S. M., Heller, W., Stewart, J. L., Silton, R. L., Edgar, J. C., Fisher, J. E., & Miller, G. A. (2010). Time course of attentional bias in anxiety: Emotion and gender specificity. *Psychophysiology*, 47(2), 247–259. doi:10.1111/j.1469-8986.2009.00926.x
- Schmukle, S. C. (2005). Unreliability of the dot probe task. *European Journal of Personality*, 19(7), 595–605.
- Spielberger, C. D., Gorsuch, R. L., Lushene, P. R., Vagg, P. R., & Jacobs, A. G. (1983). *Manual for the State-Trait Anxiety Inventory* (Form Y). Palo Alto, CA: Consulting Psychologists Press.
- Staugaard, S. R. (2009). Reliability of two versions of the dot-probe task using photographic faces. *Psychology Science Quarterly*, 51(3), 339–350.

- Vanderhasselt, M. A., Baeken, C., Hendricks, M., & De Raedt, R. (2011). The effects of high frequency rTMS on negative attentional bias are influenced by baseline state anxiety. *Neuropsychologia*, *49*(7), 1824–1830. doi:10.1016/j.neuropsychologia.2011.03.006
- Waechter, S., Nelson, A. L., Wright, C., Hyatt, A., & Oakman, J. (2014). Measuring attentional bias to threat: Reliability of dot probe and eye movement indices. *Cognitive Therapy and Research*, *38*(3), 313–333.
- Weierich, M. R., Treat, T. A., & Hollingworth, A. (2008). Theories and measurement of visual attentional processing in anxiety. *Cognition & Emotion*, *22*, 985–1018. doi:10.1080/02699930701597601
- Zwanzger, P., Steinberg, C., Rehbein, M. A., Brockelmann, A. K., Dobel, C., Zavorotnyy, M., ... Junghofer, M. (2014). Inhibitory repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex modulates early affective processing. *NeuroImage*, *101*, 193–203. doi:10.1016/j.neuroimage.2014.07.003