Baeken, C., De Raedt, R., Van Schuerbeek, P., Vanderhasselt, M.A, De Mey, J., Bossuyt, A., & Luypaert, R. (2010). Right prefrontal HF-rTMS attenuates right amygdala's processing of negatively valenced emotional stimuli in healthy females. *Behavioural Brain Research, 214*, 450-455.

Right prefrontal HF-rTMS attenuates right amygdala processing of negatively valenced emotional stimuli in healthy females

C. Baeken[1,CA], R. De Raedt[2], P. Van Schuerbeek[3], M.A. Vanderhasselt[2], J. De Mey[3], A. Bossuyt[4], R. Luypaert[3]

1 Department of Psychiatry, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium

2 Ghent University, Department of Psychology, Ghent, Belgium

3 Department of Radiology and Medical Imaging, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium

4 Department of Nuclear Medicine, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium

---

Corresponding author with complete address, including an email address

[CA] Corresponding author: Baeken Chris, M.D., Ph.D. Department of Psychiatry, University Hospital UZBrussel, Vrije Universiteit Brussel (VUB). Laarbeeklaan 101, 1090 Brussels, Belgium. Tel: +3224776425. Fax: +32247776800. E-mail: chris.baeken@uzbrussel.be

keywords: HF-rTMS; fMRI; Lateralization; neurocircuitry; Amygdala; Healthy females

Abstract

Repetitive Transcranial Magnetic Stimulation (rTMS) studies investigating brain imaging correlates of emotion modulation in healthy volunteers can improve our understanding of the 'affective' impact of this application. In this fMRI study, we focused on lateralized amygdala functioning when processing salient emotional visual stimuli after one high-frequency (HF)-rTMS session. In a 'uniform sample' of 20 right-handed, non-depressed, healthy female subjects we examined whether one HF-rTMS session applied to the left (n=10) or right (n=10) dorsolateral prefrontal cortex (DLPFC) would influence amygdala responses to positively and negatively valenced baby faces. Subjects were given no other instructions than to focus on the emotion the visual stimuli elicited during scanning. One HF-rTMS session did not result in a conscious mood change. Whereas one left-sided HF-rTMS session did not affect amygdala processing of the positive or negative stimuli, after a single right-sided HF-rTMS session we found a significant right amygdala activity attenuation during the processing of negatively valenced baby faces. This finding provides additional evidence supporting the role of the right anterior hemisphere in the processing of negative emotional information, and increases our understanding of HF-rTMS treatment effects in mental disorders.

1. Introduction

Although repetitive Transcranial Magnetic Stimulation (rTMS) has been put forward as a promising tool to treat mental illnesses and to investigate lateralized neural connections in the living human brain [28, 43], it remains largely unclear as to how rTMS can have an influence on affect [25, 27]. As human emotion is regulated by a complex of interacting neural circuits, brain responses to emotional stimuli (e.g. emotional pictures) operate rather independently of consciously experienced mood and could provide more insight into the neurocircuitry of emotion processing [13]. In line with current brain models, which hypothesize a lateralized hemispheric specialization of emotion processing [19, 20], several rTMS studies using visual emotional stimuli found contrasting emotional reaction patterns depending on which prefrontal part of the brain they stimulated: positive or approach-related emotions were lateralized towards the left anterior hemisphere, and negative or withdrawal-related emotions towards the right anterior hemisphere [12, 18, 42]. However, these studies did not include concomitant brain imaging.

In the lateralized cortico-subcortical neuronal circuit that deals with emotional stimuli, the amygdalae play a key role in the processing of approach-related emotions, such as happiness, and in the processing of withdrawal-related emotions, such as disgust [50, 64]. They also play an important role in the regulation of attention and memory processes, they are activated during the evaluation of social cues such as facial emotional expressions, and they are implicated in 'normal' mood changes as well as in 'dysfunctional' states present in affective disorders [21, 32, 58, 62]. Although it is broadly recognized that the amygdalae are key structures in the processing of emotional information, there still remains some ambiguity in the existing affective neuroscience literature about the laterality of neural correlates of positively and negatively valenced affects. This is presumably due to the fact that several factors, such as age, gender, individual differences and task instruction can influence amygdala neuronal activities [16, 51].

As the amygdalae are thought to be under prefrontal cortical top-down control [41], the main purpose of the current fMRI study was to investigate whether

one HF-rTMS session delivered to the left or right dorsolateral prefrontal cortex (DLPFC) would influence left and right amygdala activity differently during the processing of visual stimuli with strong emotional content. We used a specially adapted paradigm with baby faces as visual 'emotional' stimuli, which were shown earlier to strongly activate the amygdalae in healthy females [4]. Positive (i.e. approach-related) stimuli were provided by smiling baby faces, negative (i.e. withdrawal-related) stimuli by baby faces exhibiting a combination of sad facial expressions and severe dermatological conditions.

As we expected that via top-down neuronal circuits left-sided HF-rTMS would predominantly affect left amygdala activity and right-sided HF-rTMS primarily right amygdala activity and in line with the anterior hemisphere specialization for approach and withdrawal-related emotions, we hypothesized that left prefrontal HF-rTMS would predominantly affect left amygdala's activity when processing positive information whereas evaluating negative stimuli, right prefrontal HF-rTMS would affect primarily right amygdala processing.

2. Materials and Methods

2.1. Participants

The study was approved by the ethics committee of the University Hospital of the Vrije universiteit Brussel (UZBrussel) in accordance with the guidelines for medical research involving human subjects laid down in the Declaration of Helsinki in 1964. All study subjects gave written informed consent and were financially compensated.

As age and gender differences could confound brain imaging results, twenty healthy, right-handed medication-free female volunteers were recruited to participate in this study. The mean age of participants was 23.30 years (*SD*=2.94). All subjects were naïve to the rTMS method and were randomized into two groups. One group received one real HF-rTMS session applied to the left DLPFC (n=10), the second group received one real HF-rTMS session applied to the right DLPFC (n=10). Before inclusion in the study, participants were carefully screened. No medication, except birth-control pills, was allowed. Psychiatric disorders were excluded using the Mini-International Neuropsychiatric Interview (Mini [52]) and the Dutch translation of the Beck Depression inventory (BDI [7]). Female volunteers with a psychiatric disorder and/or a score higher than eight on the BDI were excluded. Right-handedness was assessed with the Van Strien Questionnaire [57]. Additionally, participants completed a Dutch version of The Profile of Mood States (POMS [59]), a 32-item inventory that assesses five mood dimensions, just before (T_1) and just after each HF-rTMS session (T_2), as well as just after each fMRI scan (T_3). Feelings of ‘depression’ (8 items: minimum score 0; maximum score 32), ‘fatigue’ (6 items: minimum score 0; maximum score 24), ‘tension’ (6items: minimum score 0; maximum score 24), ‘anger’ (7 items: minimum score 0; maximum score 28) and ‘vigor’ (5 items: minimum score 0; maximum score 20) were rated. Ratings were made on four-point scales: a rating of 0 indicates a low level and a rating of 4 indicating a high level of a given mood.

2.2. Repetitive transcranial magnetic stimulation (rTMS).

For the application of rTMS we used a Magstim high-speed magnetic stimulator (Magstim Company Limited, Wales, UK), connected to a specially designed figure-of-eight-shaped coil. Before each application, the motor threshold (MT) of each individual was determined. Single pulse TMS in combination with motor evoked potentials (MEP), measured with surface electromyography (EMG) registration determined the optimal muscular response (right abductor pollicis brevis muscle). We adjusted stimulus intensity until positive MEP responses were recorded and clear thumb muscular abduction was observed. Before the selected part of the cortex was accepted as the motor cortex related to the contralateral APB muscle, positive MEP responses of at least 50 μV (peak-to-peak amplitude) had to be produced in at least five out of ten consecutive trials. A stimulation intensity of 110 % of the subject's resting MT was used. In order to target the left and right DLPFC (i.e. the centre part of the midprefrontal gyrus (Brodmann 9/46)) accurately, the precise stimulation site and position of the coil were determined using MRI non-stereotactic guidance [44]. Perpendicular to this point, the precise stimulation site on the skull was marked and stimulated. In each high-frequency (10 Hz) stimulation session, subjects received forty trains of 3.9 seconds duration, separated by an intertrain interval of 26.1 seconds. Each session, therefore, lasted 20 minutes (1560 pulses per session). Subjects wore earplugs and were blindfolded. The International Society of Transcranial Magnetic Stimulation (ISTS) safety guidelines were followed [2, 60].

2.3. Stimuli

As described in Baeken et al [4], twenty female volunteers (mean age=25.8 year, *SD*=5.9), unrelated to the imaging part of this study and of the same socio-cultural background, evaluated a series of baby faces (90 color images of emotional baby faces: 40 positive, 40 negative and 10 neutral; mean estimated age of the babies=5.50 months, *SD*=4.00) with a paper and pencil version of the Self-Assessment Manikin (SAM), using a 9 point likert type scale for ratings of valence and arousal [9]. Out of

these ‘baby face’ pictures we chose only those positively or negatively valenced images that were equal in arousal levels. Eight positively valenced (mean valence=7.71, *SD*=0.23: mean arousal=6.50, *SD*=0.27), 8 negatively valenced (mean valence=2.22, *SD*=0.36: mean arousal= 6.76, SD=0.50) and 8 neutral (mean valence=4.81, *SD*=0.20: mean arousal=1.52, *SD*=0.23) baby face pictures were selected to be used for the imaging study (for an example, see Fig 1A). Independent *t*-tests confirmed that the valence dimensions of the selected positive and negative pictures were significantly different (t (14)=36.52, $p<0.001$), but not the arousal dimensions (t (14)=1.32, $p=0.21$). This adapted paradigm with baby faces as visual ‘emotional’ stimuli was chosen to evaluate approach and withdrawal-related emotions. Studies indicate that infant faces elicit stronger emotional responses than those of adults [8]. From an ‘evolutionary’ perspective, such stimuli would strongly engage attention [16] and should induce spontaneous emotional reactions in female subjects [5]. To avoid emotional ambiguity and to elicit withdrawal-related emotions in women, we used baby faces exhibiting a combination of negative facial expressions and severe ‘dermatological’ conditions as negative stimuli.

2.4. Paradigm

A ‘single’ blind, between-subjects design was used. All volunteers were stimulated within the same time schedule, between 10 am and 14 pm noon. Just before and 20 minutes after the HF-rTMS session (delay including mood assessment) all subjects underwent the baby face paradigm in the MRI scanner. In healthy subjects George et al. [26] demonstrated that serum cortisol mean levels increase slightly for 30 minutes after right and left prefrontal stimulation and subsequently decline, indicating that the neurobiological effects of one rTMS session should be measured within this time frame. During fMRI scanning, stimuli followed a blocked design with 19 blocks of 20 sec (with 4 pictures) each. To control for carryover effects between conditions, the blocks were organized in a counterbalanced fashion (neutral-negative-positive-neutral-positive-negative-neutral-negative-positive-

neutral-positive-negative-neutral-negative-positive-neutral-positive-negative-neutral).

Images were displayed for 4.5 seconds and preceded by a short flash of a black crosshair centered on a white background, introduced for fixation purposes. Each positive, negative and neutral image occurred three times in this sequence. Subjects were given no other instructions than to focus on the emotion the visual stimuli elicited.

2.5. Image acquisition and data analysis

The study was carried out on a 1.5T MRI scanner (Philips Intera, Best, The Netherlands) equipped with a 6 channel sense head coil. We measured 127 consecutive FFE-EPI volumes (TR/TE=3000/35 msec, flip angle=90°, 18 slices, slice thickness/gap=5.0/1.0 mm, size=64x64, in plane resolution=3.75x3.75 mm, duration 6 min 21 sec) covering the whole brain. During functional magnetic resonance imaging, pictures that varied in emotional content (positive, negative or neutral valence) were back-projected onto a flat screen positioned at the subject's feet and were viewed via a mirror mounted on the head coil. After the fMRI experiment, a T1-weighted structural scan (3D IR-TFE, TI/TR/TE=1501/16/4.6 msec, flip angle=30°, matrix=256x256, in plane resolution=1.0x1.0 mm, 100 slices, slice thickness 2.0 mm, duration 6 min 24 sec) of the whole head was performed.

The fMRI data were analyzed with SPM5 software (Wellcome Department of Cognitive Neurology, London, UK). The fMRI time series were realigned to their first volume to correct for head movements. After the realignment step, normalization into the standard anatomical space (EPI MNI template) and smoothing with an 8 mm Gaussian kernel were performed. The anatomical scan was normalized to the standard anatomical space (T1 MNI template) to be used as anatomical underlay for the results.

The effects under consideration were estimated using the general linear model [23]. We modeled our 3 regressors of interest, positive, negative and neutral, as separate boxcar functions convolved with the canonical HRF. For each subject, we

generated contrast (percentage signal change) maps and t-statistic maps corresponding to the contrasts positive versus neutral and negative versus neutral. Starting from the individual contrast maps we performed a 1-sample *t*-test for each contrast. The *t*-statistic maps of this random effects analysis were thresholded at p (uncorrected)<0.001.

In a following step, to provide statistical evidence of lateralized amygdala functioning in relation to positive and negative emotional experiences, a ROI analysis was performed in Marsbar [11]. First of all, we used the AAL-atlas (Anatomical Automatic Labelling [55]) to define two mask-volumes which masked the whole brain with the exception of the left or right amygdala respectively. To define our ROI's, we started from masked t-maps. To take inter-subject variability into account, we adjusted the threshold for the activation pixels for each subject using a technique that was introduced earlier by a number of other authors in order to improve the robustness of lateralization measures [22, 33, 35]. For each amygdala, we calculated a mean maximum *t* defined as the mean of the 5% highest *t*-values. The final ROIs contained the voxels in the masked maps with a t-value above 20% of the mean maximum *t*. This approach selected those voxels which were most significantly activated within each amygdala. In a following step, we calculated the mean contrast value for positive vs .neutral and negative vs. neutral contrasts within each ROI for each subject individually and this for the left and right amygdala separately. All further statistical analyses were performed in SPSS 15 (SPSS, Chicago, IL).

To analyze the effect of one left and right HF-rTMS session on the processing of the emotional visual stimuli, we performed a repeated measures ANOVA analysis for the left and right amygdala activity separately, with HF-rTMS (before vs. after HF-rTMS) and picture valence (positive vs. negative) as within-subject factors, and site (left-sided real HF-rTMS vs. right-sided real HF-rTMS) as between-subjects factor. The significance threshold for all SPSS analyses was set at a two- tailed probability of $p \leq 0.05$.

3. Results

3.1. Behavioral results.

POMS ratings were analyzed for the left and right group separately, using multivariate ANOVA with each POMS subscale as dependent variable and time (T_1, T_2 and T_3) as fixed factor. See Table 1.

For the group of healthy female volunteers receiving left-sided HF-rTMS, the MANOVA for the POMS-depression subscale showed no significant difference for time ($F(2,29)=0.17$, $p=0.85$). The MANOVA for the POMS-anger subscale did not reveal significant effects for time ($F(2,29)=2.01$, $p=0.15$), and neither did the MANOVA's for tension (anxiety) ($F(2,29)=0.42$, $p=0.66$), fatigue ($F(2,29)=1.13$, $p=0.34$), and vigor ($F(2,29)=2.84$, $p=0.08$).

Concerning the group receiving right-sided HF-rTMS, the performed MANOVA's did not show significant differences for time for the POMS subscales of depression ($F(2,29)=0.46$, $p=0.64$), anger ($F(2,29)=0.50$, $p=0.61$), tension ($F(2,29)=0.29$, $p=0.75$), fatigue ($F(2,29)=1.05$, $p=0.36$), and vigor ($F(2,29)=0.10$, $p=0.90$).

3.1. Brain imaging results.

See Table 2. The Kolmogorov-Smirnov normality test on left and right amygdala activity failed to show normality (p's< 0.05). Logarithmic transformation resulted in data normality (p's> 0.05). Firstly, when analyzing the repeated measures ANOVA for the evaluation of the left amygdala responses to the baby faces, we found no main effect of HF-rTMS ($F(1, 18)= 0.14$, $p=0.71$) or valence ($F(1,18)=0.04$, $p=0.84$). The main effect for site was also not significant ($F(1,18)=0.03$, $p=0.86$). Moreover, the interaction effect between HF-rTMS and valence was not significant ($F(1, 18)=0.11$, $p=0.74$) nor between HF-rTMS and site ($F(1, 18)=1.92$, $p=0.18$) or between valence and site ($F(1, 18)= 0.14$, $p=0.71$). The

three-way interaction between HF-rTMS, valence and site was also not significant (F(1, 18)= 0.11, p= 0.75; η^2 =0.01, observed power estimate= 0.06).

Secondly, when analyzing the repeated measures ANOVA for right amygdala responses to the salient stimuli, again we found no main effect of HF-rTMS (F(1,18)=0.30, p=0.59) or valence (F(1,18)<0.01, p=0.95). The main effect for site was not significant (F(1,18)=0.24, p=0.63). The interaction effect between HF-rTMS and site was also not significant (F(1, 18)=1.06, p=0.32), nor between valence and site (F(1, 18)=0.10, p=0.75), or between HF-rTMS and valence (F(1, 18)=1.20, p=0.29). However, the crucial three-way interaction between HF-rTMS, valence and site was significant (F(1, 18)=4.62, p<0.05; η^2 =0.21, observed power estimate= 0.53). Post hoc paired t-tests revealed that for the volunteers receiving right-sided stimulation, after viewing negatively valenced baby faces, right amygdala activity significantly decreased (t(9)=2.76, p=0.02). No pre-post significance was reached for the processing of positively valenced baby faces (t(9)=0.35, p=0.74). No pre-post differences were observed for the group of females receiving left-sided HF-rTMS when evaluating the positively valenced baby faces (t(9)=0.25, p=0.81) nor when evaluating the negatively valenced babies (t(8)=0.49, p=0.64).

4. Discussion

First of all, one HF-rTMS session did not result in a conscious mood change in the healthy females of both groups studied. Although both groups were relatively small, this finding corresponds to previous reports of single sham-controlled sessions of HF-rTMS over the left or right DLPFC, causing no immediate or delayed mood changes in comparable groups of healthy female volunteers [3]. Secondly, our random effects analysis (Fig 1) confirmed our earlier results of amygdalae neuronal involvement when processing positively and negatively valenced baby faces [4].

However, the aim of the present study was to further elucidate the lateralized role of the 'affective' amygdalae within a normal cortico-limbic circuit, manipulating their emotional processing by using HF-rTMS. Contrary to our initial hypothesis, where we expected that left prefrontal HF-rTMS would predominantly affect left amygdala activity when processing positive information, no changes in this area was observed. One left-sided HF-rTMS session also did not affect amygdalae processing for negatively valenced stimuli. These results indicate that in healthy females, with a 'normal' functioning neuronal circuit, one left dorsolateral prefrontal HF-rTMS session does not alter amygdalae processing of emotional visual stimuli. As no immediate improvements in the attentional processing of emotional information following one session of HF-rTMS applied to the left DLPFC were found, either in healthy females [37] or depressed individuals [38], one HF-rTMS session applied to the left DLPFC in healthy subjects might rather influence general cognitive information processing [56] (for an overview see [31]).

In line with the right-sided hemispheric specialization hypothesis on the processing withdrawal-related emotions, we found following right prefrontal HF-rTMS a significant attenuation of right amygdala activity only when evaluating negatively valenced stimuli. No right-sided HF-rTMS impact on amygdalae activity was observed for the processing of positively valenced baby faces. As no left amygdala changes were detected after right-sided HF-rTMS, our results indicate that one such session did not influence sustained emotional reactions neither in the processing of positive information nor in the evaluation of negative stimuli. The

current findings of right amygdala attenuation of neuronal activity when processing negatively valenced facial expressions following right dorsolateral prefrontal HF-rTMS can be interpreted within a cortico-limbic network that plays a central role in the processing of emotional visual stimuli. The initial 'disruption' of neural processes under the stimulation coil post HF-rTMS is in general not only followed by increased neuronal activities in these areas but neuronal alterations are as well observed in other prefrontal cortical areas, such as the anterior cingulate cortex (ACC), and in subcortical limbic structures, such as the amygdalae [34, 36, 39, 43]. Current hypotheses on the working mechanisms of rTMS state that the influence of rTMS occurs at the subcortical level, such as the hypothalamus, suggesting that the prefrontal cortex participates in the rTMS-induced blunted stress response of hypothalamic-pituitary-adrenocortical (HPA) system-activity [48]. Interestingly, the neurobiological modulation of stress responses seems to be lateralized to the right prefrontal cortex [14]. Animal models also indicate that stress-regulatory systems such as the HPA-axis are predominantly regulated by the right prefrontal cortex [54].

Altogether, our results suggest that our initial hypothesis could be correct: in healthy women right-sided prefrontal HF-rTMS increased the top-down regulatory effect of the prefrontal cortices, thereby influencing subcortical systems implicated in affective appraisal, which in turn can lead to reduced withdrawal-related mood over time. Although a dorsomedial prefrontal cortex-amygdala top-down circuit in fear extinction under normal and stressful conditions has been proposed [1], at this point it remains speculative to assume that the observed attenuation of right amygdala activity after HF-rTMS is the result of (re)appraisal or suppression processes, diminishing the negative impact of an aversive event [30, 41]. Nevertheless, it has been demonstrated earlier that the activation of the DLPFC inhibited negative affects in combination with amygdala attenuation while viewing highly aversive visual stimuli [45]. At this point, it's worth pointing out two interesting trends in Table 2: the neural activity in the left amygdala was higher in response to positive and negative stimuli after left-sided HF-TMS, and the neural

activity in the left amygdala was lower in response to positive and negative stimuli after right-sided HF-TMS.

The finding of immediate attenuation of the processing of negative ('aversive') information following one session of HF-rTMS applied over the right DLPFC might be especially germane in light of the therapeutic applications of rTMS as a treatment for posttraumatic stress disorder (PTSD), a severe psychiatric disorder that can develop after exposure to any psychological trauma [15]. Amygdala hyper-responsiveness in PTSD patients has been reported after exposure not only to traumatic cues but also to trauma-unrelated emotional material such as fearful facial expressions [52]. Besides HPA-system alterations [63], dysfunctional neuronal top-down circuits in PTSD patients such as the ACC and the mediodorsal prefrontal cortical areas, have been implicated in failing to inhibit abnormal amygdalae functioning [46]. Whereas Rosenberg and colleagues [49] reported that left-sided HF-rTMS treatment in PTSD patients primarily resulted in beneficial global mood changes and not in core trauma symptom changes, Cohen et al [15] reported on alleviation of anxiety symptoms after right–sided HF-rTMS treatment. It has to be noticed that in our sample no female volunteers were diagnosed with PTSD and of course it remains questionable whether rTMS effects observed in healthy volunteers can be transferred to neurobiological rTMS effects found in psychiatric patients [65].

Due to the relatively small sample size a regions-of-interest approach including only the left and right amygdala was selected. No other brain areas were investigated. Consequently, at this point, it remains speculative to assume that the attenuation of right-sided amygdala activity post right-sided HF-rTMS was directly initiated by a modified unilateral/bilateral DLPFC activity. Moreover, POMS mood assessment was performed before and after, but not during, the fMRI procedure. Because we did not ask our participants to rate their mood during the fMRI experiment it remains to some extent speculative to attribute the neuronal changes to alterations in emotional processes. However, the main reason as to why we did not include a 'forced cognitive task' in our paradigm - for instance by requiring an explicit emotional judgment about the images presented, or asking subjects to rate

their personal change in ‘feeling’ when watching the different blocks – was to evaluate ‘spontaneous’ brain responses when 'mentalizing emotional feelings without the ‘contamination’ of merely cognitive neuronal processes. A possible weakness of the study is the fact that we did not use any kind of manipulation check (such as eye movement monitoring). One could argue that female volunteers did not pay attention to intrinsically aversive nature of the negative stimuli. However, this would have been the same for the group of healthy females receiving one left sided HF-rTMS session. Furthermore, the instruction to focus on the emotional experience that the images of babies elicited does take subject involvement beyond ‘passive’ viewing.”

Whether (re)appraisal/ suppression processes initiated by prefrontal or by subcortical circuits are the essential mediators of the observed attenuation effect on the emotional amygdala remains a critical question for future research. It could be argued that the observed attenuation of right-sided amygdala activity is the result of habituation processes. Several authors report that repetitive presentation of visual stimuli can result in decreased metabolic activity in both amygdalae [10, 47, 61]. However, if habituation processes would have been involved, the group of volunteers receiving left-sided stimulation should also have shown decreases in right-sided amygdala activity. Furthermore, the attenuation of right-sided amygdala activity post HF-rTMS was only present when processing withdrawal-related emotional stimuli, and decreases in amygdala activity predominantly occur during viewing happy facial expressions or viewing faces of loved ones [6].

Although the current experiment was limited by sample size, an important strength of this study was the choice of using 3D-MRI to locate the target site, which allowed us to objectively know that the DLPFC was stimulated and not, for instance, premotor or ventrolateral cortical areas. Because emotional valence and arousal may be controlled by different neural systems [22, 29], another major advantage of this study lies in the fact that the positive or negative emotional stimuli were chosen to be equal for arousal.

In conclusion, in line with the lateralized anterior hemisphere specializations for withdrawal-related emotions, our observations suggest that after

the evaluation of negatively valenced visual stimuli one HF-rTMS session results in a specific effect on right amygdala activity. These findings provide additional evidence supporting the role of the right anterior hemisphere - including the amygdalae - in the processing of negative emotional information. Although our results could be indicative as to why right-sided HF-rTMS can influence the processing of withdrawal-related emotions, future research should replicate this design in a sham-controlled gender and age mixed sample in which every subject would have been participated in one left and right-sided HF-rTMS session and it would be of interest to evaluate these kinds of brain imaging paradigms in combination with rTMS treatment procedures for PTSD.

Acknowledgements:

This research was supported by a grant from the Scientific Fund W. Gepts UZBrussel. The authors wish to acknowledge the contributions of P. Marchand, M.D. of the Department of Paediatrics (UZBrussel) for estimating the age of the depicted babies.

References

[1] Akirav I, Maroun M. The role of the medial prefrontal cortex-amygdala circuit in stress effects on the extinction of fear. Neural Plast, 2007;30873.

[2] Anand S, Hotson J. Transcranial magnetic stimulation: neurophysiological applications and safety. Brain Cogn, 2002; 50: 366-386.

[3] Baeken C, Leyman L, De Raedt R, Vanderhasselt MA, D'haenen H. Left and right High Frequency repetitive Transcranial Magnetic Stimulation of the dorsolateral prefrontal cortex does not affect mood in female volunteers. Clin Neurophysiol, 2008; 119:568-575.

[4] Baeken C, De Raedt R, Ramsey N, Van Schuerbeek P, Hermes D, Bossuyt A, Leyman L, Vanderhasselt MA, De Mey J, Luypaert R. Amygdala responses to positively and negatively valenced baby faces in healthy female volunteers: influences of individual differences in harm avoidance. Brain Res, 2009;1296:94-103.

[5] Baeken C, Van Schuerbeek P, De Raedt R, Bossuyt A, Vanderhasselt MA, De Mey J, Luypaert R. Passively viewing negatively valenced baby faces attenuates left amygdala activity in healthy females scoring high on 'Harm Avoidance' Neurosci Lett, 2010 (in press).

[5] Bartels A, Zeki S. The neural correlates of maternal and romantic love. Neuroimage, 2004;21: 1155-1166.

[6] Beck AT, Steer RA. Internal consistencies of the original and revised Beck Depression Inventory. J. Clin. Psychol, 1984;40: 1365-1367.

[7] Best CT, Womer JS, Queen HF. Hemispheric asymmetries in adults' perception of infant emotional expressions. J Exp Psychol Hum Percept Perform, 1994;20: 751-765.

[8] Bradley, M.M. & Lang, P.J.. Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. Journal of Behavior Therapy and Experimental Psychiatry, 1994;25: 49-59.

[9] Breiter HC, Etcoff NL, Whalen PJ, Kenedy WA, Rauch SL, Buckner RL, Strauss MM, Hyman SE and Rosen BR. Response and habituation of the human amygdala during visual processing of facial expression. Neuron, 1996;17: 875–887.

[10] Brett M, Anton J-L, Valabregue R, Poline J-B.. Region of interest analysis using an SPM toolbox [abstract]. Presented at the Eighth International Conference of Functional Mapping of

the Human Brain, June 2–6, 2002, Sendai, Japan. Available on CD-ROM in NeuroImage, Vol. 16, No. 2.

[11] Brüne M, Bahramali H, Hennessy M, Snyder A. Are angry male and female faces represented in opposite hemispheres of the female brain? A study using repetitive transcranial magnetic stimulation (rTMS). J Integr Neurosci, 2006;5:187-197.

[12] Buck R. The biological affects: a typology. Psychol. Rev, 1999;106: 301-336.

[13] Cerqueira JJ, Almeida OF, Sousa N. The stressed prefrontal cortex. Left? Right! Brain Behav Immun, 2008;22:630-638.

[14] Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, Grisaru N. Repetitive transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in posttraumatic stress disorder: a double-blind, placebo-controlled study. Am J Psychiatry, 2004; 161:515-524.

[15] Compton RJ. The interface between emotion and attention: a review of evidence from psychology and neuroscience. Behav Cogn Neurosci Rev, 2003;2: 115-129.

[16] Costafreda SG, Brammer MJ, David AS, Fu CH.Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PET and fMRI studies.Brain Res Rev, 2008;58:57-70.

[16] d'Alfonso AA, Van Honk J, Hermans E, Postma A, De Haan EH. Laterality effects in selective attention to threat after repetitive transcranial magnetic stimulation at the prefrontal cortex in female subjects. Neurosci Lett, 2000;280: 195-198.

[17] Davidson RJ, Jackson DC, Kalin NH. Emotion, plasticity, context, and regulation: perspectives from affective neuroscience. Psychol Bull, 2000; 126: 890-909.

[18] Demaree HA, Everhart DE, Youngstrom EA, Harrison DW. Brain lateralization of emotional processing: historical roots and a future incorporating "dominance".Behav Cogn Neurosci Rev, 2006; 4:3-20.

[19] Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr Opin Neurobiol, 2001; 11:240-249.

[20] Fernández G,de Greiff A, von Oertzen J, Reuber M, Lun S, Klaver P, Ruhlmann J, Reul J, Elger CE. Language mapping in less than 15 minutes: real-time functional MRI during routine clinical investigation, NeuroImage, 2001;14 : 585–594.

[21] Friston KJ, Harrison L, Penny W. Dynamic causal modelling. Neuroimage, 2003; 19: 1273-1302.

[22] Garavan H, Pendergrass JC, Ross TJ, Stein EA, Risinger RC. Amygdala response to both positively and negatively valenced stimuli. Neuroreport, 2001;12: 2779-2783.

[23] Garcia-Toro M, Montes JM, Talavera JA. Functional cerebral asymmetry in affective disorders: new facts contributed by transcranial magnetic stimulation. J Affect Disord, 2001; 66:103-109.

[24] George MS, Wassermann EM, Williams WA, Steppel J, Pascual-Leone A, Basser P, Hallett M, Post RM. Changes in mood and hormone levels after rapid-rate transcranial magnetic stimulation (rTMS) of the prefrontal cortex. J Neuropsychiatry Clin Neurosci, 1996;8:172-180.

[25] George MS, Avery D, Nahas Z, Molloy M, Oliver NC, Risch SC, Arana GW. rTMS studies of mood and emotion. Electroencephalogr. Clin. Neurophysiol Suppl, 1999; 51: 304-314.

[26] George MS, Nahas Z, Kozol FA, Li X., Yamanaka K, Mishory A, Bohning DE. Mechanisms and the current state of transcranial magnetic stimulation. CNS Spectr, 2003;8: 496-514.

[27] Gerber AJ, Posner J, Gorman D, Colibazzi T, Yu S, Wang Z, Kangarlu A, Zhu H, Russell J, Peterson BS. An affective circumplex model of neural systems subserving valence, arousal, and cognitive overlay during the appraisal of emotional faces. Neuropsychologia, 2008; 46: 2129-2139.

[29] Goldin PR, McRae K, Ramel W, Gross JJ. The neural bases of emotion regulation: reappraisal and suppression of negative emotion. Biol Psychiatry, 2008; 63:577-586.

[31] Guse B, Falkai P, Wobrock T. Cognitive effects of high-frequency repetitive transcranial magnetic stimulation: a systematic review. J Neural Transm, 2010; 117: 105-122.

[32] Irwin W, Anderle MJ, Abercrombie HC, Schaefer SM, Kalin NH, Davidson RJ. Amygdalar interhemispheric functional connectivity differs between the non-depressed and depressed human brain. Neuroimage, 2004; 21: 674-686.

[33] Jansen A, Menke R, Sommer J, Förster AF, Bruchmann S, Hempleman J, Weber B, Knecht S. The assessment of hemispheric lateralization in functional MRI--robustness and reproducibility, Neuroimage, 2006;33: 204-217.

[34] Kimbrell TA, Dunn RT, George MS, Danielson AL, Willis MW, Repella JD, Benson BE, Herscovitch P, Post RM, Wassermann EM. Left prefrontal-repetitive transcranial magnetic stimulation (rTMS) and regional cerebral glucose metabolism in normal volunteers. Psychiatry Res, 2002;115: 101-113.

[35] Knecht S, Jansen A, Frank A, van Randenborgh J, Sommer J, Kanowski M, Heinze HJ. How atypical is atypical language dominance? Neuroimage, 2003; 18: 917-927.

[36] Knoch D, Treyer V, Regard M, Muri RM, Buck A, Weber B. Lateralized and frequency-dependent effects of prefrontal rTMS on regional cerebral blood flow. Neuroimage, 2006;31:641-648.

[37] Leyman L, De Raedt R, Vanderhasselt MA, Baeken C. Influence of high-frequency repetitive transcranial magnetic stimulation over the dorsolateral prefrontal cortex on the inhibition of emotional information in healthy volunteers. Psychol Med, 2009;39:1019-1028.

[38] Leyman L, De Raedt R, Vanderhasselt MA & Baeken C. Effects of repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex on the attentional processing of emotional information in major depression: A pilot study. Psychiatry Research, in press.

[39] Nahas Z, Lomarev M, Roberts DR, Shastri A, Lorberbaum JP, Teneback C, McConnell K, Vincent DJ, LI X, George M, Bohning DE. Unilateral Left Prefrontal Magnetic Stimulation (TMS) Produces Intensity-Dependent Bilateral Effects as Measured by Interleaved BOLD fMRI. Biol Psychiatry, 2001;50: 712-720.

[40] Ochsner KN, Ray RD, Cooper JC, Robertson ER, Chopra S, Gabrieli JD, Gross JJ. For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. Neuroimage, 2004;23: 483-499.

[41] Ochsner KN, Ray RR, Hughes B, McRae K, Cooper JC, Weber J, Gabrieli JD, Gross JJ. Bottom-Up and Top-Down Processes in Emotion Generation: Common and Distinct Neural Mechanisms. Psychol Sci, in press.

[42] Padberg F, Juckel G, Prassl A, Zwanzger P, Mavrogiorgou P, Hegerl U, Hampel H, Moller HJ. Prefrontal cortex modulation of mood and emotionally induced facial expressions: a transcranial magnetic stimulation study. J. Neuropsychiatry Clin. Neurosci, 2001;13: 206-212.

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

[44] Peleman K, Van Schuerbeek P, Luypaert R, Stadnik T, De Raedt R, De Mey J, Bossuyt A and Baeken C. Using 3D-MRI to localize the Dorsolateral Prefrontal Cortex in TMS Research. The World Journal of Biological Psychiatry, 2010; 11(2 Pt 2):425-430.

[45] Phan KL, Fitzgerald DA, Nathan PJ, Moore GJ, Uhde TW, Tancer ME. Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. Biol Psychiatry, 2005; 57:210-219.

[46] Phan KL, Britton JC, Taylor SF, Fig LM, Liberzon I. Corticolimbic blood flow during nontraumatic emotional processing in posttraumatic stress disorder. Arch Gen Psychiatry, 2006; 63:184-192.

[47] Posamentier MT & Abdi H. Processing faces and facial expressions. Neuropsychol Rev, 2003; 13: 113-143.

[48] Post A, Keck, ME Transcranial magnetic stimulation as a therapeutic tool in psychiatry: what do we know about the neurobiological mechanisms? J. Psychiatr Res, 2001;35:193-215.

[49] Rosenberg PB, Mehndiratta RB, Mehndiratta YP, Wamer A, Rosse RB, Balish M. Repetitive transcranial magnetic stimulation treatment of comorbid posttraumatic stress disorder and major depression. J Neuropsychiatry Clin Neurosci, 2002;14: 270-276.

[50] Schienle A, Schäfer A, Hermann A, Walter B, Stark R, Vaitl D. fMRI responses to pictures of mutilation and contamination. Neurosci Lett, 2006;393:174-178.

[51] Sergerie K, Chochol C, Armony JL. The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev, 2008;32:811-830.

[51] Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the

development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry, 1998;59: 22-33.

[52] Shin LM, Rauch SL, Pitman RK, Whalen PJ. The human amygdala in anxiety disorders. In the human amygdala. (Eds) Whalen PJ & Phelps EA.The Guilford Press, NY London. 299, 2009; pp 321-343.

[54] Sullivan RM, Gratton A Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. Psychoneuroendocrinology, 2002;27:99-114.

[55] Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage, 2002; 15: 273-289.

[56] Vanderhasselt MA, De Raedt R, Baeken C, Leyman L, D'haenen H.. The influence of rTMS over the left dorsolateral prefrontal cortex on Stroop task performance. Exp Brain Res, 2006; 169:279-282.

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

[58] Wager TD, Phan KL, Liberzon I, Taylor SF. Valence, gender, and lateralization of functional brain anatomy in emotion: a meta-analysis of findings from neuroimaging. Neuroimage, 2003; 19: 513-531.

[59] Wald FD, Mellenbergh GJ. De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). Nederlands tijdschrift voor de psychologie, 1990; 45: 86-90.

[60] Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr. Clin. Neurophysiol, 1998; 108: 1-16.

[61] Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA. Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci, 1998;18: 411-418.

[62] Yang TT, Menon V, Eliez S, Blasey C, White CD, Reid AJ, Gotlib IH, Reiss AL Amygdalar activation associated with positive and negative facial expressions. Neuroreport, 2002; 13: 1737-1741.

[63] Yehuda R. Biology of posttraumatic stress disorder. J Clin Psychiatry (2001) 17:41-46.

[65] Zald DH.The human amygdala and the emotional evaluation of sensory stimuli. Brain Research: Brain Research Reviews, 2003; 41: 88-123.

[66] Zwanzger P, Fallgatter AJ, Zavorotnyy M, Padberg F. Anxiolytic effects of transcranial magnetic stimulation--an alternative treatment option in anxiety disorders? J Neural Transm, 2009; 116:767-775.

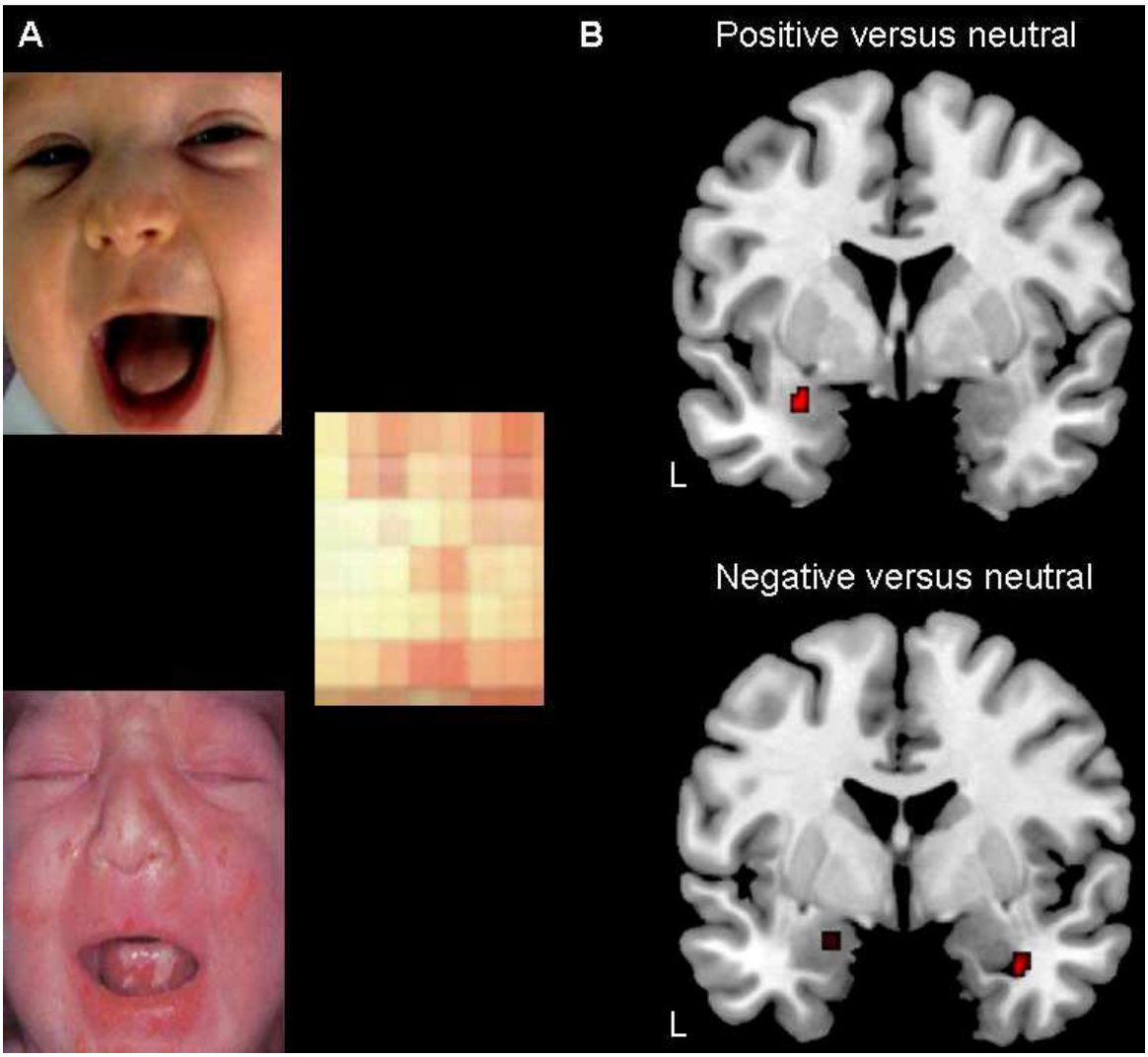

Fig 1: A) Example of a 'positive', a 'neutral' and a 'negative' picture. B) Coronal view of amygdalae activity found in a random effects analysis (T-contrast, p= 0.005, uncorrected; k> 10 voxels) for the whole group of healthy women before receiving left or right-sided HF-rTMS overlaid on an anatomical T1-image for upper) the positive>neutral contrast (left amygdala: x=-28, y=2, z=-16) and lower) the negative>neutral contrast (left amygdala: x=-20, y=-4, z=-20; right amygdala: x=36, y=0 z=-26). (L=left). The red areas represent the significantly activated clusters in the left and/or right amygdala.

Left	**Profile of Mood States**				
	Depression	Anger	Tension	Fatigue	Vigour
Before HF-rTMS	0.1 (0.3)	0.5 (1.0)	0.8 (1.9)	2.2 (2.8)	10.4 (3.3)
After HF-rTMS	0.2 (0.6)	0.0 (0.0)	1.0 (2.2)	2.1 (2.7)	11.5 (3.9)
After fMRI	0.1 (0.3)	0.1 (0.3)	0.3 (0.9)	4.2 (4.7)	7.9 (3.2)
p-values	0.85	0.15	0.66	0.34	0.08
Right	Depression	Anger	Tension	Fatigue	Vigour
Before HF-rTMS	0.1 (0.3)	0.5 (0.8)	0.7 (1.5)	1.7 (1.8)	8.0 (4.4)
After HF-rTMS	0.3 (0.9)	1.2 (2.1)	0.7 (1.3)	0.9 (1.0)	7.1 (4.3)
After fMRI	0.5 (1.3)	0.7 (1.6)	1.1 (1.3)	1.9 (2.0)	7.7 (4.9)
p-values	0.64	0.61	0.75	0.36	0.90

Table 1: Means and standard deviations for each Profile of Mood States subscale for the left and right stimulated group separately. The presented p-values represent the MANOVA results for each POMS subscale separately ($p \leq 0.05$, two- tailed).

Left	**Profile of Mood States**				
	Depression	Anger	Tension	Fatigue	Vigour
Before HF-rTMS	0.1 (0.3)	0.5 (1.0)	0.8 (1.9)	2.2 (2.8)	10.4 (3.3)
After HF-rTMS	0.2 (0.6)	0.0 (0.0)	1.0 (2.2)	2.1 (2.7)	11.5 (3.9)
After fMRI	0.1 (0.3)	0.1 (0.3)	0.3 (0.9)	4.2 (4.7)	7.9 (3.2)
p-values	0.85	0.15	0.66	0.34	0.08
Right	Depression	Anger	Tension	Fatigue	Vigour
Before HF-rTMS	0.1 (0.3)	0.5 (0.8)	0.7 (1.5)	1.7 (1.8)	8.0 (4.4)
After HF-rTMS	0.3 (0.9)	1.2 (2.1)	0.7 (1.3)	0.9 (1.0)	7.1 (4.3)
After fMRI	0.5 (1.3)	0.7 (1.6)	1.1 (1.3)	1.9 (2.0)	7.7 (4.9)
p-values	0.64	0.61	0.75	0.36	0.90

Table 1: Means and standard deviations for each Profile of Mood States subscale for the left and right stimulated group separately. The presented p-values represent the MANOVA results for each POMS subscale separately ($p \leq 0.05$, two- tailed).