doi:10.1093/scan/nss074 SCAN (2013) 8, 828–837

Self-referential processing influences functional activation during cognitive control: an fMRI study

Gerd Wagner,[1] Kathrin Koch,[1] Claudia Schachtzabel,[1] Gregor Peikert,[1] Carl Christoph Schultz,[1] Jürgen R. Reichenbach,[2] Heinrich Sauer,[1] and Ralf G. Schlösser[1]

[1]Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena, Germany and [2]Medical Physics Group, Institute of Diagnostic and Interventional Radiology I, Jena University Hospital, Jena, Germany

Rostral anterior cingulate cortex (rACC) plays a central role in the pathophysiology of major depressive disorder (MDD). As we reported in our previous study (Wagner *et al.*, 2006), patients with MDD were characterized by an inability to deactivate this region during cognitive processing leading to a compensatory prefrontal hyperactivation. This hyperactivation in rACC may be related to a deficient inhibitory control of negative self-referential processes, which in turn may interfere with cognitive control task execution and the underlying fronto-cingulate network activation. To test this assumption, a functional magnetic resonance imaging study was conducted in 34 healthy subjects. Univariate and functional connectivity analyses in statistical parametric mapping software 8 were used. Self-referential stimuli and the Stroop task were presented in an event-related design. As hypothesized, rACC was specifically engaged during negative self-referential processing (SRP) and was significantly related to the degree of depressive symptoms in participants. BOLD signal in rACC showed increased valence-dependent (negative *vs* neutral SRP) interaction with BOLD signal in prefrontal and dorsal anterior cingulate regions during Stroop task performance. This result provides strong support for the notion that enhanced rACC interacts with brain regions involved in cognitive control processes and substantiates our previous interpretation of increased rACC and prefrontal activation in patients during Stroop task.

Keywords: fMRI; rostral anterior cingulate; cognitive control; emotion; self-referential processing; functional connectivity; major depression

INTRODUCTION

The rostral anterior cingulate cortex (rACC) is involved in controlling affective states and plays a major role in the psychopathology of major depressive disorder (MDD). In healthy controls, it has been found to be of major relevance in tasks involving self-reflection, such as reflecting about one's own future (Sharot *et al.*, 2007), thinking about hopes and aspirations (Johnson *et al.*, 2006) and during sad mood induction using autobiographical scripts (Liotti *et al.*, 2000).

In patients with MDD, the activity of rACC has been often shown to be altered in the resting state condition as well as during performance of cognitive and affective tasks (Wagner *et al.*, 2006; Drevets *et al.*, 2008; Walter *et al.*, 2009; Yoshimura *et al.*, 2010). This aberrant activity in rACC seems to be associated with depressed mood and cognitive impairments. Moreover, depressed patients are often characterized by difficulties in disengaging from self-focusing and inhibiting context-irrelevant self-related information, e.g. negative self-relevant thoughts, which in turn may lead to disturbed attention in cognitively demanding tasks (Siegle *et al.*, 2002; Joormann and Gotlib, 2008). These difficulties have been related to a putative dysfunction in rACC and adjacent ventromedial prefrontal cortex.

Furthermore, rACC represents a central node within a brain network subserving processing of emotional and motivational stimuli. This brain network often seems to 'deactivate' during cognitive tasks, which has led to the suggestion that self-reflective thoughts may be a common 'default mode', when individuals are not otherwise engaged (Gusnard *et al.*, 2001). All of the putative functions in the 'default mode network' (DMN), such as beliefs, remembering the past as well as planning the future are self-referential in nature (Buckner *et al.*, 2008). A failure in the inhibition of these processes may lead to an interference with cognitive task performance, as often seen in patients with depression.

Received 24 October 2011; Accepted 18 July 2012
Advance Access publication 13 July 2012

This research was supported by the German Federal Ministry of Education and Research (BMBF, grant 01GW0740).

Correspondence should be addressed to Gerd Wagner, Department of Psychiatry and Psychotherapy, Jena University Hospital, Centre for Neuroimaging, Jahnstr. 3, 07740 Jena, Germany. E-mail: wagner.gerd@uni-jena.de

In agreement with this assumption, neuropsychological studies provided evidence that depressive subjects had deficient cognitive inhibition and enhanced interference sensitivity when performing tasks tapping cognitive control functions (Ottowitz *et al.*, 2002). The Stroop-Color-Word Test (Stroop task; Stroop, 1935) is an established neuropsychological task taxing inhibitory cognitive control and has been consistently shown to activate the fronto-cingulate network (Peterson *et al.*, 1999). Therefore, in order to investigate the neural basis of cognitive inhibition processes in patients with MDD, we used the Stroop task in our previous functional magnetic resonance imaging (fMRI) study (Wagner *et al.*, 2006). The main finding was that unmedicated depressed patients were unable to suppress rACC activation during Stroop task performance in contrast to healthy controls. We interpreted this result in terms of an inability of patients to inhibit affective interferences, which may arise from the enhanced self-referential processing (SRP) during the execution of the Stroop task. However, the postulated interfering effect of enhanced SRP due to the increased activation in DMN and especially in rACC on cognitive control processes has not been explicitly investigated yet. To specifically test this assumption, we conducted this fMRI study in healthy controls, in which we used affective self-referential statements referring to dysfunctional depressive thinking as well as non-affective neutral statements and the cognitive Stroop task. Based on our previous study, the main hypothesis was that negative SRP is associated with an increased sustained activation in rACC, which leads to an increased fronto-cingulate activation during the Stroop task performance. To test this hypothesis, we investigated the network associated with the activation in rACC during SRP and during the Stroop task with univariate and functional connectivity fMRI analyses.

MATERIALS AND METHODS

Subjects

Thirty-four subjects (age: mean = 24.1; standard deviation (s.d.) = 6.35; range: 18–51; 28 females) recruited from the Friedrich Schiller University community participated in the experiment.

All participants were screened for the presence of current psychiatric disorder using the German version of the Mini-International Neuropsychiatric Interview (Sheehan *et al.*, 1998) and a semi-structured interview, by which neurological and past psychiatric diseases were assessed. None of the subjects fulfilled the criteria of Major Depression Disorder according to the International Classification of Diseases 10th revision.

None of the subjects reported a present or past history of drug abuse. Subjects had no present or past history of neurological or other clinically significant disorders. The participants were right-handed according to the modified version of the Annett handedness inventory (Briggs and Nebes, 1975) and reported normal or corrected-to-normal vision. Informed written consent was obtained in accordance with the protocols approved by the ethics committee of the University of Jena prior to conducting the study and all subjects received an allowance of 10 Euro per hour in return for their participation. Depressive symptoms were assessed with the Becks Depression Inventory (BDI) in order to relate them to brain activation in rACC. Subject had a mean BDI score of 4.91 with s.d. = 4.51 ranging from 0 to 16. In two participants, there were missing values regarding the BDI scores. According to the German version of BDI, a total score of over 18 indicates possible depression and warrants an additional clinical evaluation as confirmation (Hautzinger *et al.*, 1994). Therefore, none of the participants in this study showed clinically relevant symptoms of depression. The variation in BDI scores in this study can therefore be considered as a normal variation in symptoms assessed by BDI.

Paradigm design

SRP task

The paradigm, which was used in this study, was developed and validated in a pilot study with 20 inpatients with MDD and 20 matched healthy controls. Based on the pilot study, negative and positive self-referential statements were chosen, which were able to discriminate between depressive patients and healthy controls. Moreover, neutral self-referential statements were used in this study, which did not significantly differ between patients with MDD and healthy controls in this pilot study. Affective self-referential statements were drawn from a variety of sources, including adaptation of Velten's mood induction statements (Velten, 1968) and the Cognitive Triad Inventory (Beckham *et al.*, 1986). Parts of these statements were used in previous studies of our group to induce negative affect in healthy subjects as well as in patients with MDD (Terhaar *et al.*, 2009; Wagner *et al.*, 2009; Köbele *et al.*, 2010). Thus, we used 20 negative self-referential statements in this study dealing with negative view about the own self, e.g. 'I consider myself to be a loser', and 20 positive self-referent statements related to the positive view about the own self, such as 'I have a lot of positive qualities'. Finally, 20 neutral statements were presented describing one's traits or attitudes such as 'I prefer to spend money instead of saving it'. Subjects were asked to judge positive, negative and neutral self-referential statements on a four-point scale, as to whether they properly described the participants themselves. All stimuli were matched according to the word number and length (no significant differences in the mean number) as well as syntax.

Stroop task

The manual version of the Stroop task was described in detail in our previous article (Wagner *et al.*, 2006). In brief, the Stroop task consisted of two conditions: a congruent and an incongruent condition. In the congruent condition, color words were presented in the color denoted by the corresponding word; in the incongruent condition, color words were displayed in one of three colors not denoted by the word. This target stimulus was presented in the center of the display screen. Two possible answers (color words in black type) were presented below it (in the lower visual field) in order to minimize contextual memory demand. The subjects had to indicate as fast as possible the type of color by pressing one of two buttons (with index or middle finger), which corresponded spatially to both possible answers.

Paradigm timing

The whole paradigm consisted of 60 self-referential statements and 60 Stroop stimuli, which were presented in a pseudorandomized order and combination: each of 10 negative, 10 positive and 10 neutral self-referential stimuli were combined with each of 30 congruent Stroop stimuli to one trial, the other 10 negative, 10 positive and 10 neutral self-referential stimuli were combined with 30 incongruent Stroop stimuli. The detailed timing of the paradigm is illustrated in Figure 1. To minimize cognitive processes and brain activations due to task-switching costs, we introduced, prior to the presentation of each self-referential statement and Stroop stimulus, a task-specific cue for a short time (Verbruggen *et al.*, 2007). Furthermore, to guarantee that subjects concentrated on the reading of the statements, the self-referential statement was presented during the first 3.5 s without and during the last 3 s with a digit bar with four possible responses. To be able to separate BOLD signal due to processing of self-referential from Stroop stimuli, a variable fixation baseline from 6 to 8 s with temporal jittering was introduced within each single trial. Single trials were separated by a variable temporally jittered fixation baseline varying from 6.5 to 11 s in duration.

The whole paradigm was implemented using Presentation software (http://www.neurobs.com/) running on a PC, which was connected to a video projector. The visual stimuli were projected on a transparent screen inside the scanner tunnel, which could be viewed by the subject through a mirror system mounted on top of the MRI head coil. The subjects' responses were registered by an MRI-compatible fiber optic response device (Lightwave Medical Industries, Canada) with four buttons on a keypad for the right hand.

MRI parameters

Functional images were recorded on a 3 Tesla whole-body scanner (MAGNETOM Trio, A Tim System; Siemens, Erlangen, Germany) with a 12-channel head matrix coil by using a whole-brain T2*-weighted Echo Planar Imaging (EPI) sequence, covering a volume of 48 parallel slices with 2.7 mm slice thickness and an isotropic voxel size of $2.7 \times 2.7 \times 2.7\,mm^3$. Repetition Time (TR) was 2700 ms, Echo Time (TE) 30 ms, the flip angle was $\alpha = 90^\circ$ and the Field Of View (FOV) was 192 mm × 192 mm (matrix 72×72). Parallel imaging (GRAPPA) with acceleration factor of 2 and 30 reference lines was used. One run with 600 volume acquisitions was collected from each participant. In addition, a high-resolution structural scan was acquired for co-registration using a three-dimensional (3D) Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence with 192 contiguous axial slices of 1 mm thickness (TR 2300 ms; TE 3 ms; flip angle 9°; matrix size 256×256; isotropic voxel dimensions of $1 \times 1 \times 1\,mm^3$).

Functional data analyses

Data preprocessing

The first four EPI images were discarded from further analysis to avoid T1 saturation effects. The functional images were preprocessed with the statistical parametric mapping software (SPM8, Wellcome Department of Cognitive Neurology, London, UK). Pre-processing included slice timing correction using the middle image of the volume as reference slice and 3D motion correction, i.e. rigid body realignment to the mean of all images. It was ensured that head movement was below 3 mm and 3° for each participant. Subsequently,

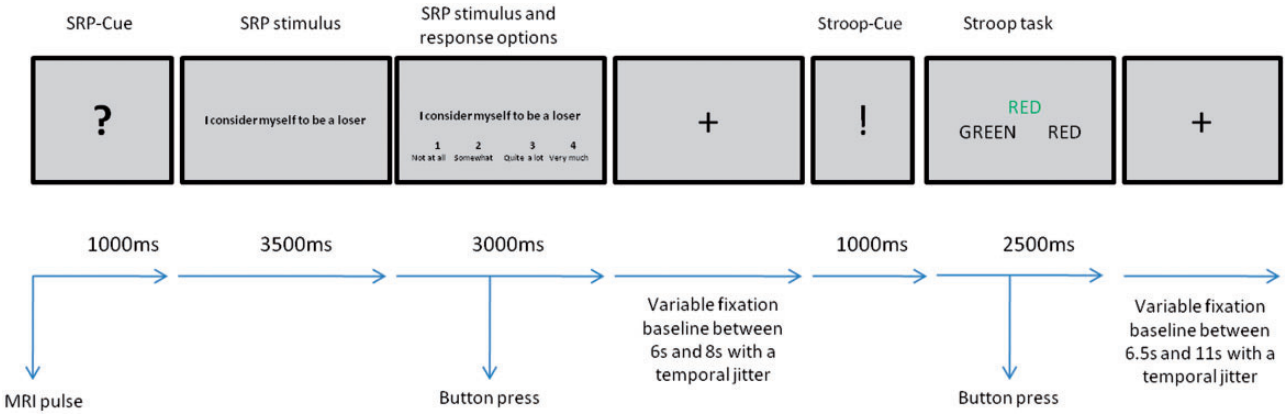

Fig. 1 Experimental task design and timing.

within-subject registration was performed between functional and anatomical images. The co-registered anatomical images were segmented using tissue probability maps of the International Consortium for Brain Mapping (ICBM) template in SPM8. Functional images were then spatially normalized to the Monteal Neurological Institute (MNI) space using spatial normalization parameters estimated during the segmentation process.

The data were smoothed using a Gaussian filter of 8 mm Full Width at Half Maximum (FWHM) and a temporal high-pass filter of 128 s was applied to remove low-frequency confounds. The first-level analysis of the imaging data was conducted using an event-related design. Regressors of interest represented the three levels of valence (negative, positive and neutral) and six Stroop task × valence combinations. To control for the potential confound of subjects' movement during the task, individual movement parameters were entered as covariates into the design matrix as estimated during the realignment step. All regressors were convolved with a model of the Haemodynamic Response Function (HRF). Contrast images for each single regressor against baseline were calculated for each subject.

Statistical analysis

Univariate analysis

The single-subject contrasts were submitted to the second-level group analyses with subject as the random-effect variable. For the analysis of the brain networks involved in SRP, a one-way analysis of variance (ANOVA) with three levels of the factor VALENCE, i.e. negative, positive and neutral self-related items, was performed. Two different contrasts were of interest: comparison of the negative SRP trials with the neutral SRP trials as well as of the positive with neutral SRP trials.

To investigate the brain network involved in the interaction between SRP and Stroop task, a two-way ANOVA with the first factor VALENCE with three levels (negative, positive and neutral) and the second factor TASK with two levels (congruent and incongruent Stroop task condition) were set up.

Activations were identified as significant if they passed a height threshold of $P < 0.001$ uncorrected for multiple comparisons at the whole brain level and a spatial extent threshold according to the expected number of voxels per cluster. Furthermore, due to our strong hypothesis regarding the role of cingulate cortex and predominantly rACC in SRP, we used a mask image of the whole cingulate cortex, which we created by means of the WFU Pickatlas (http://fmri.wfubmc.edu/) to restrict the analysis in the second step to the cingulate cortex only. This analysis was corrected for multiple comparisons using the Family Wise Error (FWE) correction. All MNI coordinates were converted to Talairach coordinates using the *mni2tal* algorithm (Brett *et al.*, 2001).

Functional connectivity (psychophysiological interaction)

To assess the hypothesis that enhanced activation in rACC during negative SRP interacts with fronto-cingulate brain regions during Stroop task performance, functional connectivity analysis was conducted using the psychophysiological interactions (PPIs) analysis methodology as used in a previous study of our group (Schlosser *et al.*, 2009) and as implemented in SPM8. Each PPI analysis was individually computed for each subject and the contrast images derived from these analyses were then entered into the group-level one-sample *t*-test, thresholded at $P < 0.001$, uncorrected. In more detail, at the individual subject level, for each subject, an average time course was extracted from the seed region of interest (rACC), defined as a 4 mm sphere around coordinates derived from the individual local maximum coordinate within the rACC. For this purpose, we first created a mask image as a 10 mm radius sphere around the maximum coordinate ($x = 8$, $y = 34$ and $z = 10$) from the contrast negative *vs* neutral SRP condition. Subsequently, the individual local maximum within this mask image was extracted to build the individual seed Region of Interest (ROI). The mean MNI coordinates of the rACC were $x = 7.7$, $y = 31.8$ and $z = 19.9$. The time series extracted from this ROI was adjusted for the effects of interest, i.e. mean-corrected according to the session mean. We then constructed an individual design matrix with one regressor representing the activation time course in the rACC, one regressor representing the time-dependent change as a psychological variable of interest (from the design matrix) and a third regressor representing the element-by-element product of the previous two (the PPI term). As the psychological variable, the difference between Stroop-related activation after negative self-referential stimuli and Stroop-related activation after neutral self-referential stimuli was used. With this implementation of the PPI analysis, significant SPM activations of a particular area would reflect changes in functional connectivity between the source area (i.e. rACC) and the activated regions associated with the Stroop task depending on prior valence of SRP.

RESULTS

Behavioral results

SRP task

The mean score for negative SRP items was mean = 1.49 (s.d. = 0.38), for positive SRP items M = 3.16 (s.d. = 0.46) and for the neutral SRP items mean = 2.77 (s.d. = 0.18). These responses were significantly different from each other ($P < 0.0001$), indicating a significant difference

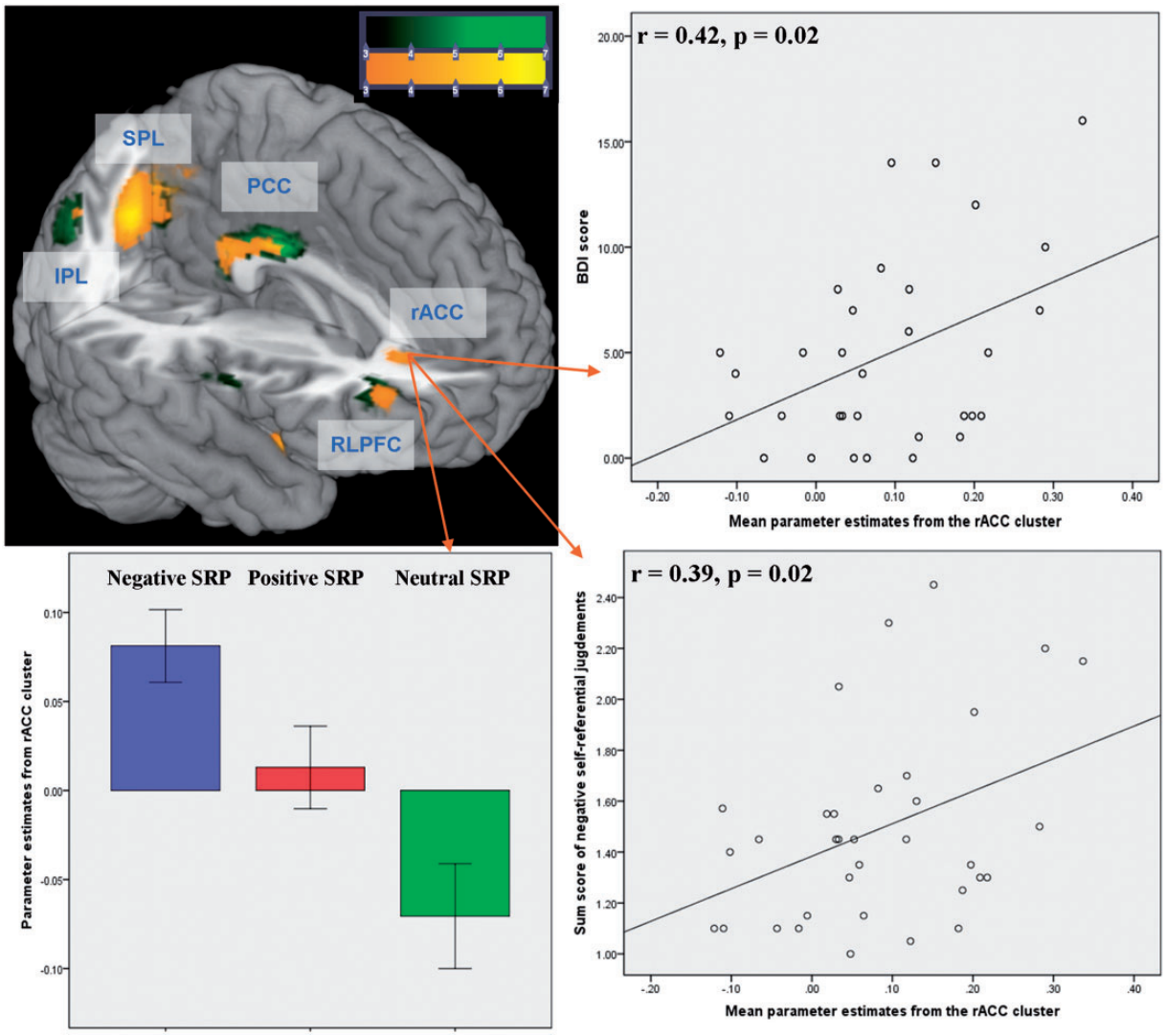

Fig. 2 Brain regions showing significant BOLD signal difference comparing negative (yellow color) and positive (green color) with neutral self-referential statements ($P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster). The error bars represent standard error of the mean. The scatter plots depict the relationship between BDI, negative SRP judgments and parameter estimates from the rACC. IPL, inferior parietal lobe.

in response patterns between neutral and affective items as well as between positive and negative items.

Responses for negative SRP items negatively correlated with responses for positive SRP items ($r = -0.79$, $P < 0.0001$). Furthermore, negative SRP score positively correlated with the BDI score ($r = 0.79$, $P < 0.0001$), indicating a good criterion validity of SRP items to measure depression-specific attitudes.

Stroop task

The two-way ANOVA with the within-subject factors TASK (incongruent *vs* congruent Stroop condition) and VALENCE (Stroop task after negative *vs* positive *vs* neutral SRP conditions) revealed for the reaction time (RT), a significant main effect of TASK [$F(1,33) = 217.35$, $P < 0.0001$] indicating slower performance in the incongruent compared with the congruent condition. There was no significant main effect of VALENCE, but a TASK × VALENCE interaction [$F(2,66) = 5.52$, $P = 0.006$]. *Post hoc t*-tests revealed significantly prolonged RT for the congruent condition after presentation of negative compared with neutral SRP items ($t = 3.03$, $P = 0.005$) and significantly prolonged RT for the incongruent condition after presentation of neutral compared with positive SRP items ($t = 2.19$, $P = 0.035$).

In both Stroop conditions, high levels of accuracy were obtained in subjects. They showed in the incongruent condition after negative SRP items the worst performance in terms of the mean percentage of correct responses mean = 96.6% (s.d. = 5.38) and in the congruent condition after negative SRP items the best performance with mean = 98.8 % (s.d. = 3.50) correct responses. There were no significant differences between single Stroop conditions with regard to the number of correct responses.

fMRI results

Processing of negative SRP items vs neutral SRP items

As depicted in Figure 2 and Table 1, subjects activated stronger during processing of negative SRP items in contrast to neutral SRP items, predominantly in the midline brain structures including lingual gyrus, posterior cingulate cortex (PCC), precuneus/superior parietal lobe (SPL) and the right rACC. Further brain areas involved were the right rostrolateral prefrontal cortex (RLPFC, Brodmann Area (BA)10), right inferior parietal lobe and right superior temporal gyrus (STG). Activation in the PCC and rACC both survived the FWE correction ($P < 0.05$) for multiple comparisons after applying the mask image of the whole cingulate cortex.

Table 1 Maxima of regions showing significant ($P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster) overall BOLD signal increase during processing of negative *vs* neutral self-referential items as well as positive *vs* neutral self-referential stimuli.

Region of activation	Right/left	Brodmann's area	Cluster size	Talairach coordinate			*t* value
				x	*y*	*z*	
Processing of negative SRP items > neutral SRP items							
Superior parietal lobe/precuneus	R	7	2196[a]	10	−68	37	6.73
Posterior cingulate cortex	L	23	258[a]	−4	−36	24	4.02
Lingual gyrus	L	18	168	−12	−68	0	3.98
Middle frontal gyrus	R	10	78	36	49	3	3.85
Anterior cingulate	R	24	75	8	33	8	4.13
Superior temporal gyrus	R	38	45	36	7	−15	4.42
Inferior parietal cortex	R	40	37	42	−60	44	3.72
Processing of positive SRP items > neutral SRP items							
Posterior cingulate cortex	L	23	1917[a]	0	−20	30	6.14
Superior parietal lobe/precuneus	L	7		−10	−62	33	5.4
Inferior parietal cortex	R	40	209[a]	44	−62	42	4.67
Middle frontal gyrus	L	10	199[a]	−28	45	−4	3.96
Anterior cingulate	L	32		−16	39	4	4.69
Medial frontal gyrus	R	10	164[a]	10	36	−10	4.11
Insula	L	13	146[a]	−38	3	13	4.33
Superior temporal gyrus	L	41	136[a]	−55	−26	14	4.37
Precentral gyrus	R	6	57	55	−3	9	4
Medial frontal gyrus	R	9	52	22	27	32	4.14

[a]FDR cluster level corrected.

Processing of positive SRP items vs neutral SRP items

During processing of positive SRP items in contrast to neutral SRP items, subjects showed stronger activation in similar brain structures as during processing of negative SRP items including PCC and SPL (Figure 2 and Table 1). Further cluster of stronger activation during processing of positive SRP items included the left and right RLPFC extending to the left rACC. In addition, left insular cortex, left STG and the right precentral gyrus were strongly activated in comparison to the neutral SRP condition. Both the activation in the PCC and in the left rACC survived the FWE correction ($P < 0.05$) for multiple comparisons after applying the mask image of the whole cingulate cortex. However, although the activation in rACC during processing of negative SRP items was above the fixation baseline and thus represents a difference in activation level (Figure 1), the difference between the positive and neutral SRP condition in rACC/RLPFC activation is related to differences in deactivation level.

Relationship to depressive symptoms

Testing the relationship of the rACC activation during negative SRP condition with the degree of depressive symptoms, a significant positive correlation was detected between parameter estimates from the rACC and BDI scores ($r = 0.42$, $P = 0.016$) as well as with the sum score of negative self-referential judgments ($r = 0.39$, $P = 0.019$) as depicted in Figure 2. Parameter estimates drawn from other clusters of the contrast SRP negative *vs* SRP neutral, e.g. from the PCC or from the RLPFC, were not significantly correlated with BDI scores indicating the specificity of the relationship between rACC activation and depressive symptoms.

Effects of SRP on brain activation during the Stroop task

When testing the effect of prior presentation of negative SRP items on brain activation during Stroop task performance, we observed a significant main effect of VALENCE (negative *vs* neutral SRP) in the left rACC (BA 32, $x = -14$, $y = 39$, $z = 4$, $t = 4.91$ and $k = 62$), in PCC (BA 31, $x = 0$, $y = -53$, $z = 27$, $t = 3.97$ and $k = 253$), in the right inferior frontal gyrus (BA 47, $x = 36$, $y = 9$, $z = -17$, $t = 3.82$ and $k = 30$) and in the right precentral gyrus (BA 6, $x = 55$, $y = -3$, $z = -11$, $t = 3.89$ and $k = 31$) as illustrated in Figure 3. Only the activation in the rACC survived the FWE correction ($P < 0.05$) for multiple comparisons after applying the mask image of the whole cingulate cortex as well as across the whole brain. A significant TASK (congruent *vs* incongruent) by VALENCE (negative *vs* neutral SRP) interaction was only observed in the right STG (BA 21, $x = 56$, $y = -14$, $z = -1$, $t = 3.85$ and $k = 38$).

Comparing both Stroop conditions after presentation of positive and negative SRP items, significantly higher Stroop task activations were detected in the left insula (BA 13, $x = -30$, $y = 6$, $z = 5$, $t = 3.72$, $k = 33$) after positive SRP items and significantly higher Stroop task activation in the left STG (BA 21, $x = -48$, $y = -25$, $z = -4$, $t = 4.19$, $k = 29$) after negative SRP items.

A significant TASK × VALENCE (negative *vs* positive) interaction was observed resulting predominantly in activations of ACC as well as bilaterally of the ventrolateral and RLPFC as depicted in Figure 4 and Table 2. The ACC activations passed the correction for multiple comparisons ($P < 0.05$) using the mask image of the cingulate cortex. This comparison indicates a BOLD signal increase from the congruent to incongruent condition after negative SRP condition in contrast to BOLD signal decrease from the incongruent to congruent condition after positive SRP condition (Figure 4). The opposite interaction contrast revealed no significant voxels.

Functional connectivity: PPI analysis

In order to examine the main hypothesis of the interfering effect of enhanced rACC activity during negative SRP on cognitive control brain areas, the PPI analysis revealed that during Stroop task performance rACC activity significantly interacted with predominantly left fronto-cingulate brain regions, i.e. with the dorsal ACC and the Ventrolateral Prefrontal Cortex (VLPFC) as well as with the left temporal and occipital gyrus in dependence on the VALENCE (negative *vs* neutral) of SRP before Stroop task presentation (Figure 5 and Table 3). The Dorsal Anterior Cingulate Cortex (dACC) cluster survived the cluster-level correction for multiple comparisons (FWE, $P < 0.05$) after masking with the whole cingulate cortex, although there was only a trend (FWE, $P = 0.06$) for FWE corrected statistical significance on the voxel-wise level.

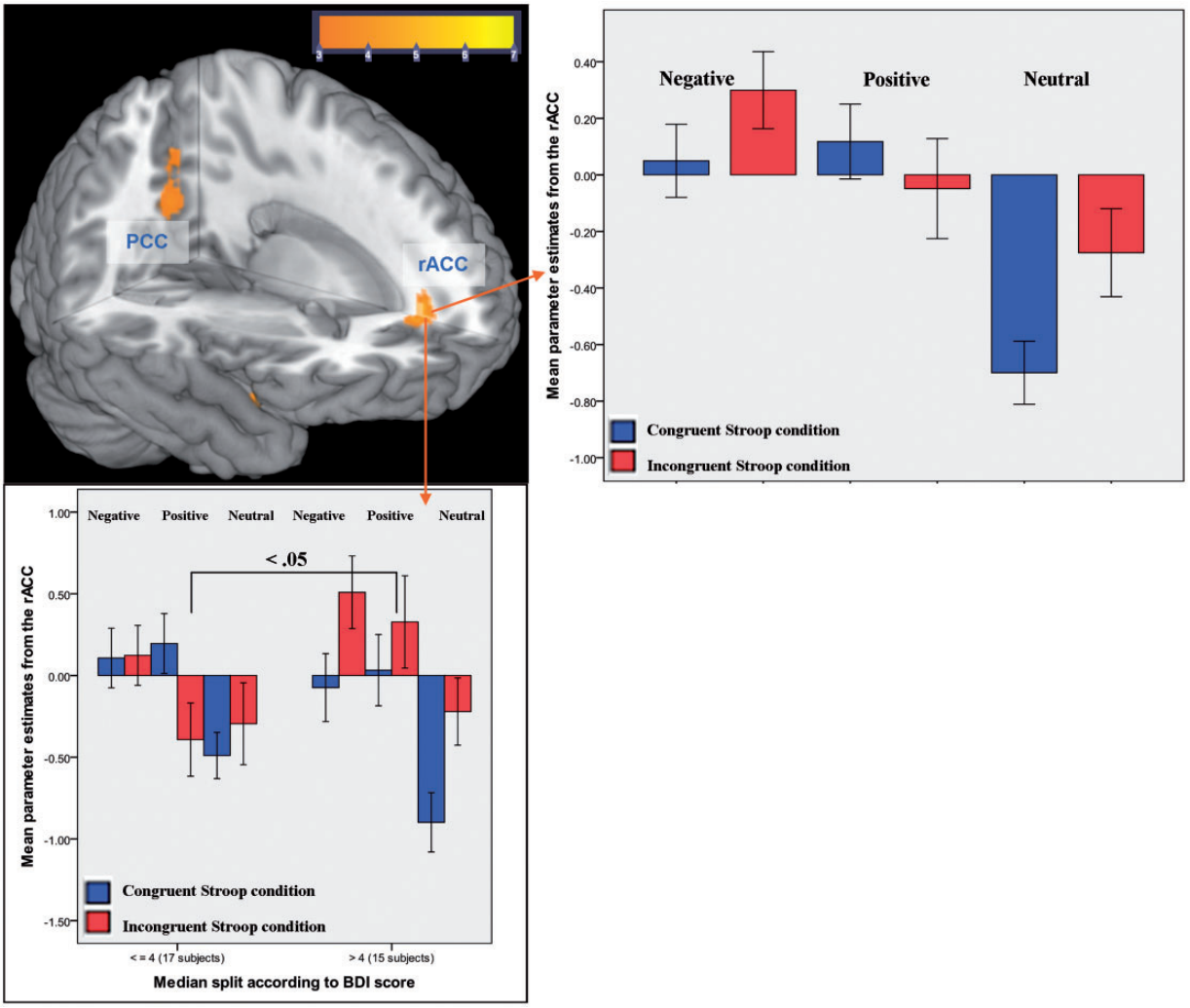

Fig. 3 Brain regions showing significant BOLD signal difference comparing both Stroop conditions after presentation of negative *vs* neutral SRP statements (main effect of valence for Stroop task; $P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster). The lower bar graph separately displays parameter estimates from the rACC cluster for both groups of healthy subjects split according to the median BDI value (BDI = 4). The error bars represent standard error of the mean.

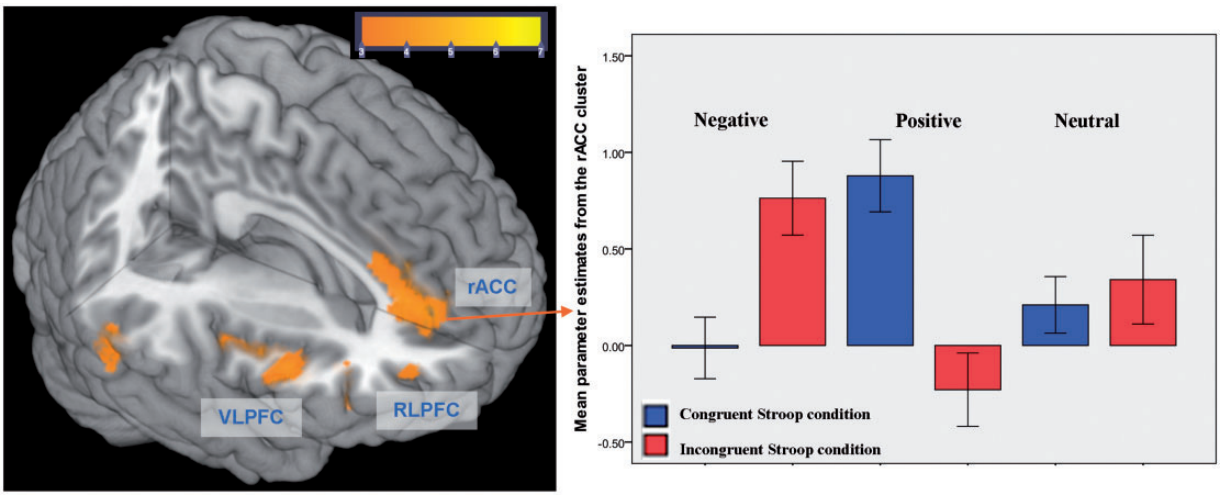

Fig. 4 Brain regions showing significant BOLD signal in the TASK (incongruent *vs* congruent Stroop conditions) by VALENCE (presentation after negative *vs* positive SRP items) interaction ($P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster). The error bars represent standard error of the mean.

Table 2 Maxima of regions showing significant ($P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster) significant BOLD signal in the TASK (congruent *vs* incongruent Stroop conditions) by VALENCE (presentation after negative *vs* positive SRP items) interaction.

Region of activation	Right/left	Brodmann's area	Cluster size	Talairach coordinate			*t* value
				x	*y*	*z*	
Anterior cingulate	L	24	649[a]	−6	28	15	4.49
	R	32		2	43	11	4.32
Inferior frontal gyrus	R	45/47	605[a]	48	20	3	4.11
Superior frontal gyrus	R	10	448[a]	34	51	12	4.95
Inferior parietal lobe	R	40	255[a]	55	−45	26	3.82
Middle temporal gyrus	R	21	113	63	−37	2	3.83
Inferior frontal gyrus	L	47	102	−42	14	−6	4
Middle frontal gyrus	L	10	77	−34	45	12	3.57

[a]FDR cluster level corrected.

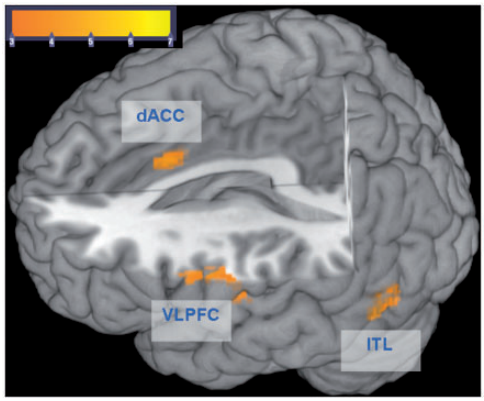

Fig. 5 PPI analysis: regions showing significant task-dependent (Stroop task after negative vs. after neutral SRP items) interaction with the rACC ($P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster).

DISCUSSION

In this study, we investigated functional brain correlates of affective and non-affective SRP and its impact on subsequent cognitive control processes. Our main focus was on the examination of the role of rACC during SRP and its hypothesized interfering influence on brain activation during subsequent Stroop task. As expected, we found that rACC was specifically engaged during negative SRP and was significantly related to the degree of depressive symptoms in study participants. Moreover, BOLD signal in rACC was correlated with BOLD signal in prefrontal and dorsal anterior cingulate regions during Stroop task performance depending on the SRP valence. This result provides a strong support for the aforementioned hypothesis that enhanced rACC interacts with brain regions involved in cognitive control processes. Moreover, it substantiates our previous interpretation of increased rACC and DLPFC activation in patients with MDD during Stroop task (Wagner *et al.*, 2006) in terms of enhanced negative self-referential processes and associated prefrontal hyperactivation. This could be interpreted as a compensatory mechanism in order to maintain the normal level of task performance due to increased demands in cognitive inhibition and monitoring processes through affective interferences.

Furthermore, the functional connectivity analysis is in agreement with previous findings of our group (Schlosser *et al.*, 2008) in which an enhanced Stroop task-related input from the dorsal to rACC was observed in subjects with MDD. Thus, we could potentially demonstrate a possible neurofunctional model of depressive pathophysiology in healthy subjects by providing strong evidence for the central role of rACC in SRP and its interfering influence on cognitive processing.

SRP in healthy subject

SRP was defined in this study as a concept, in which affective and non-affective information referring to oneself is processed and a decision is made regarding oneself. The majority of studies investigating self-referential processes compared self- with other-referential stimuli and found evidence for strong involvement of cortical midline structures, i.e. PCC, ventromedial (VMPFC, overlapping with rACC) and dorsomedial PFC (DMPFC) during processing of self-related information (Northoff *et al.*, 2006; van der Meer *et al.*, 2010). For example, Johnson *et al.* (2002) compared judgments about one's own abilities, traits and attitudes to a semantic judgment task. The self-referential condition was associated with activation in VMPFC, DMPFC and PCC relative to the control condition. D'Argembeau *et al.* (2005) observed that VMPFC was more active during the self-referential than during other reflective tasks. A more differentiated view on brain structures involved in self- *vs* other-referential processing was reported in a recent review of van der Meer *et al.* (2010). The authors included 20 PET or fMRI studies on SRP into a meta-analysis and observed that the rACC was a key structure in processing self-related in contrast to other-related stimuli. The authors further suggested that rACC might be strongly involved in processing of affective self-relevant information. Moreover, according to this meta-analysis, brain activation in DMPFC and PCC did not seem to be specific for distinguishing self- *vs* other-reflection processes. DMPFC may be important in the cognitive evaluation of the self-relatedness of a stimulus, whereas PCC may be responsible for the integration of autobiographical information regarding the 'self'. However, only very few studies explicitly investigated neurofunctional differences as a function of affective and non-affective self-relevant processing. Thus, by comparing self-referential statements of different valence, present findings extend previous work on SRP pointing out the anterior and posterior cingulate cortices during affective SRP. These results provide further support for the notion that DMPFC activation, which did not differ comparing affective with non-affective self-referential stimuli, may encode the self-relevance of information regardless of the valence. The higher PCC activation during processing of positive and negative SRP stimuli in contrast to neutral statements might be explained by the higher involvement of autobiographical information during processing of affective *vs* non-affective stimuli.

The new finding is that the rACC was differentially activated depending on the valence of the SRP stimuli. One possible explanation of the enhanced rACC activation during SRP of negative stimuli is that rACC specifically encodes negative valence of self-related stimuli. However, Sharot *et al.* (2007) reported enhanced rACC activation during processing of positive in contrast to negative future expectations. Moran *et al.* (2006) did not observe differences in rACC

Table 3 Psychophysiological interaction (PPI) analysis: maxima of regions showing significant task-dependent (Stroop task after negative *vs* after neutral SRP) interaction with the rACC ($P < 0.001$ uncorrected, cluster threshold according to the expected number of voxels per cluster).

Region of activation	Right/left	Brodmann's area	Cluster size	Talairach coordinate			*t* value
				x	*y*	*z*	
Inferior frontal gyrus	L	45	126	−59	3	16	4.2
Middle temporal gyrus	L	37	102	−58	−62	−6	4.35
Anterior cingulate	L	24	76	−4	13	29	4.58
Superior temporal gyrus	R	22	37	57	0	−5	4.06
Inferior parietal lobe	L	40	26	−63	−36	28	4.11
Superior temporal gyrus	R	42	23	61	−25	9	3.92
Lingual gyrus	L	18	31	−22	−70	−10	4.19
Middle occipital gyrus	L	18	23	−46	−78	4	3.77
Middle temporal gyrus	R	21	21	44	0	−30	3.89
Lingual gyrus	R	18	18	18	−86	−4	3.82

between negative and positive self-relevant adjectives. Furthermore, a positive relationship of the degree of rACC activation to depressive symptoms in this study speaks against this narrow interpretation of a specific rACC role in detecting and processing of negative self-relevant information.

SRP in depressed patients

A more suitable interpretation might be that rACC activation may reflect the degree of attention binding on self-referential stimuli. This is in accordance with the notion of Northoff *et al.* (2006), who proposed a continuum of self-relevance and involvement for the VMPFC/rACC in coding the information for self-relevance. Because an emotional component is inherent to self-relevant processing, greater activation of the rACC indicates stronger self-relevance and stronger emotional involvement during processing of negative SRP stimuli. We therefore suggest that in this study the rACC may reflect the degree of subjective salience of self-referential stimuli by assessing emotional and autobiographical information. This is further supported by the positive correlation between the total score of negative SRP judgments, BDI score and rACC activation, indicating a stronger subjective salience of dysfunctional self-relevant depressive statements in subjects with higher manifestation of depressive symptoms. Moreover, this interpretation fits well with the core feature of depressive patients, who are characterized by strong attentional bias toward self and especially toward negative aspects of the self. In this vein, Lemogne *et al.* (2010) underlined in a recent review the importance of medial prefrontal cortex in enhanced self-focus in patients with MDD and emphasized its putative negative impact on cognitive functions due to aberrant functional connectivity between dorsal and rACC. Furthermore, recent fMRI studies of Grimm *et al.* (2011) and Cooney *et al.* (2010) have consistently reported enhanced activation in the rACC due to increased self-focus and ruminative thinking. In addition, using trait words Lemogne *et al.* (2009) observed in depressed patients increased functional connectivity between the activation cluster in the medial frontal gyrus (near the rACC) and the left DLPFC as well as dACC in the self-judgment condition. This result provides strong evidence for the interaction between the self-referential and the 'cognitive' brain network, leading potentially to impaired cognitive performance in depressive patients.

SRP and the 'DMN'

The rACC is a central part of the 'DMN,' which contains a set of medially located and interacting brain areas that are tightly functionally connected and distinct from other systems within the brain (Buckner *et al.*, 2008). It was mostly observed to be active during resting states, when mind-wandering occurs and thoughts are directed toward internal processes such as SRP. During cognitive processing of external stimuli, such as visual cues brain region within DMN have been demonstrated to decrease activity. Li *et al.* (2007) explicitly tested the postulated interplay between DMN and 'cognitive' brain network using a go/no-go paradigm. Exploring brain activity on the trials that preceded errors, the authors observed that prior to errors regions within the default network (VMPFC and PCC) showed increased activity. These data suggest that cognitive task performance may be affected when the DMN is active. Although in this study high levels of accuracy were observed during Stroop task performance in all SRP categories, significant differences in RT could be observed after negative SRP stimuli relative to neutral SRP stimuli.

On the brain activation level, a clear impact of enhanced activation in the DMN during processing of negative relative to neutral or positive self-referential stimuli on subsequent activation within the cognitive control network could be demonstrated in the univariate as well as in the functional connectivity analyses. This interfering effect seems to be strongly pronounced in subjects with a higher degree of depressive symptoms. The increased fronto-cingulate activation during Stroop task after negative SRP, as reflected in the results of the univariate and PPI analyses, may be the reason for rather moderate Stroop performance differences between single SRP valences.

These findings suggest that the inability to inhibit self-referential processes and to deactivate the DMN might be responsible for the often reported cognitive deficits in depressive patients. As we observed in our recent study (Wagner *et al.*, 2008), volumetric abnormalities in the medial Orbitofrontal Cortex (OFC) might be one major factor influencing the patients' reduced ability to deactivate the rACC and might be associated with its potentially chronically increased signal.

It should be noted that simple overlapping effect of rACC activation during SRP in terms of sustained activity on BOLD signal during Stroop task can be ruled out since there is a clear task effect with regard to the BOLD signal within the Stroop task after negative SRP condition as well as after single SRP conditions as illustrated in parameter estimates in Figures 4 and 5. Furthermore, BOLD signal in rACC during processing of self-referential stimuli was separable from the fixation baseline as illustrated in parameter estimates in Figure 2.

Moreover, it seems that during the SRP the right rACC tends to be strongly activated than during the Stroop task execution. An interesting question is whether there are functional differences in the rACC with regard to the side of activation. One explanation for the potential side differences in this study may be the choice of the statistical

threshold. Lowering the threshold to $P < 0.005$, we could observe that both the right and the left part of the rACC were activated during processing of the negative *vs* neutral self-referential statements. Another explanation may be that due to used fMRI resolution (i.e. $2.7 \times 2.7 \times 2.7\,mm^3$) and data processing strategy (e.g. spatial smoothing with 8 mm FWHM), neural activations in the left and right ACC may become at least partly indistinguishable. Thus, any reliable conclusions about the functional specialization within the left or the right rACC cannot be drawn from this study. This may explain why the majority of previous fMRI studies with depressed patients or with healthy controls using affective stimuli did not report the side of the rACC activation. First indications for potential lateralization differences in the ACC are provided by the study of Lutcke and Frahm (2008) using high-resolution fMRI. Improving the fMRI resolution may be promising to reveal potential differences in emotion regulation between the left and right ACC.

Interplay between internally and externally focused processes

Conceptualizing these results, we introduce a differentiation between internally and externally focused processes. According to Lieberman (2007), internally focused processes refer to mental processes that focus on one's own or another's mental interior, e.g. thoughts and feelings, whereas externally focused processes refer to mental processes that focus on one's own or another's physical and visible features and actions that are perceived through sensory modalities.

Evidence exists for a breakdown of the dynamic interplay between the internally and externally focused processes and the underlying brain circuits in patients with MDD. More precisely, depressed patients seem to have a strong shift in the direction of considerably increased internally focused processes, i.e. a strong shift in attention to the own self and a reduction in attention toward others and environment, which goes along with higher probability of occurrence of SRP and rumination (Joormann and Gotlib, 2008; Nolen-Hoeksema *et al.*, 2008). Previous studies observed that brain regions mainly involved in processing of internally focused information are hyperactive in patients with MDD (Greicius *et al.*, 2007; Sheline *et al.*, 2009). It was postulated that these brain areas interfere with brain regions mainly involved in processing of externally focused information, leading potentially to cognitive deficits and to difficulties in social relationships. The results of this study provide a model of how internally focused processes may interact with externally focused processes in the brain of MDD patients, emphasizing the crucial role of rACC in mediating these processes.

However, even if these data fit well with previous results in depressed patients, this model is limited by the fact that in this study only healthy subjects were investigated, who do not constitute a sample of individuals vulnerable to Major Depression. For instance, depressed patients are often characterized by structural brain changes in the fronto-cingulate network (Wagner *et al.*, 2008), which we would not expect to observe in healthy subjects. Thus, these results should be viewed with caution regarding the generalization to psychopathology of Major Depression.

It will be therefore important and promising to use the SRP paradigm for investigating the neural basis of increased self-focus in patients with MDD and its direct impact on cognitive processes. Finally, it might be a promising target biomarker for psychotherapeutic or antidepressant response prediction.

Conflict of Interest

None declared.

REFERENCES

Beckham, E.E., Leber, W.R., Watkins, J.T., Boyer, J.L., Cook, J.B. (1986). Development of an instrument to measure Beck's cognitive triad: the Cognitive Triad Inventory. *Journal of Consulting and Clinical Psychology*, *54*, 566–7.

Brett, M., Christoff, K., Lancaster, J. (2001). Using Talairach atlas with the MNI template. *Neuroimage*, *13*, S85.

Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Annals of the New York Academy of Sciences*, *1124*, 1–38.

Briggs, G.G., Nebes, R.D. (1975). Patterns of hand preference in a student population. *Cortex*, *11*, 230–8.

Cooney, R.E., Joormann, J., Eugene, F., Dennis, E.L., Gotlib, I.H. (2010). Neural correlates of rumination in depression. *Cognitive, Affective & Behavioral Neuroscience*, *10*, 470–8.

D'Argembeau, A., Collette, F., Van der Linden, M., et al. (2005). Self-referential reflective activity and its relationship with rest: a PET study. *Neuroimage*, *25*, 616–24.

Drevets, W.C., Price, J.L., Furey, M.L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Structure & Function*, *213*, 93–118.

Greicius, M.D., Flores, B.H., Menon, V., et al. (2007). Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological Psychiatry*, *62*, 429–37.

Grimm, S., Ernst, J., Boesiger, P., Schuepbach, D., Boeker, H., Northoff, G. (2011). Reduced negative BOLD responses in the default-mode network and increased self-focus in depression. *The World Journal of Biological Psychiatry*, *12*(8), 627–37.

Gusnard, D.A., Raichle, M.E., Raichle, M.E. (2001). Searching for a baseline: functional imaging and the resting human brain. *Nature Reviews Neuroscience*, *2*, 685–94.

Hautzinger, M., Bailer, M., Worall, H., Keller, F. (1994). *Beck-Depressions-Inventar (BDI)*. Bern: Huber.

Johnson, M.K., Raye, C.L., Mitchell, K.J., Touryan, S.R., Greene, E.J., Nolen-Hoeksema, S. (2006). Dissociating medial frontal and posterior cingulate activity during self-reflection. *Social Cognitive and Affective Neuroscience*, *1*, 56–64.

Johnson, S.C., Baxter, L.C., Wilder, L.S., Pipe, J.G., Heiserman, J.E., Prigatano, G.P. (2002). Neural correlates of self-reflection. *Brain*, *125*, 1808–14.

Joormann, J., Gotlib, I.H. (2008). Updating the contents of working memory in depression: interference from irrelevant negative material. *Journal of Abnormal Psychology*, *117*, 182–92.

Köbele, R., Koschke, M., Schulz, S., et al. (2010). The influence of negative mood on heart rate complexity measures and baroreflex sensitivity in healthy subjects. *Indian Journal of Psychiatry*, *52*, 42–7.

Lemogne, C., Delaveau, P., Freton, M., Guionnet, S., Fossati, P. (2010). Medial prefrontal cortex and the self in major depression. *Journal of Affective Disorders*, *136*(1–2), e1–11.

Lemogne, C., le Bastard, G., Mayberg, H., et al. (2009). In search of the depressive self: extended medial prefrontal network during self-referential processing in major depression. *Social Cognitive and Affective Neuroscience*, *4*, 305–12.

Li, C.S., Yan, P., Bergquist, K.L., Sinha, R. (2007). Greater activation of the "default" brain regions predicts stop signal errors. *Neuroimage*, *38*, 640–8.

Lieberman, M.D. (2007). Social cognitive neuroscience: a review of core processes. *Annual Review of Psychology*, *58*, 259–89.

Liotti, M., Mayberg, H.S., Brannan, S.K., McGinnis, S., Jerabek, P., Fox, P.T. (2000). Differential limbic–cortical correlates of sadness and anxiety in healthy subjects: implications for affective disorders. *Biological Psychiatry*, *48*, 30–42.

Lutcke, H., Frahm, J. (2008). Lateralized anterior cingulate function during error processing and conflict monitoring as revealed by high-resolution fMRI. *Cerebral Cortex*, *18*, 508–15.

Moran, J.M., Macrae, C.N., Heatherton, T.F., Wyland, C.L., Kelley, W.M. (2006). Neuroanatomical evidence for distinct cognitive and affective components of self. *Journal of Cognitive Neuroscience*, *18*, 1586–94.

Nolen-Hoeksema, S., Wisco, B.E., Lyubomirsky, S. (2008). Rethinking rumination. *Perspectives on Psychological Science*, *3*, 400–24.

Northoff, G., Heinzel, A., de Greck, M., Bermpohl, F., Dobrowolny, H., Panksepp, J. (2006). Self-referential processing in our brain–a meta-analysis of imaging studies on the self. *Neuroimage*, *31*, 440–57.

Ottowitz, W.E., Dougherty, D.D., Savage, C.R. (2002). The neural network basis for abnormalities of attention and executive function in major depressive disorder: implications for application of the medical disease model to psychiatric disorders. *Harvard Review of Psychiatry*, *10*, 86–99.

Peterson, B.S., Skudlarski, P., Gatenby, J.C., Zhang, H., Anderson, A.W., Gore, J.C. (1999). An fMRI study of Stroop word-color interference: evidence for cingulate subregions subserving multiple distributed attentional systems. *Biological Psychiatry*, *45*, 1237–58.

Schlosser, R., Koch, K., Wagner, G., et al. (2009). Intensive practice of a cognitive task is associated with enhanced functional integration in schizophrenia. *Psychological Medicine*, *39*, 1809–19.

Schlosser, R.G., Wagner, G., Koch, K., Dahnke, R., Reichenbach, J.R., Sauer, H. (2008). Fronto-cingulate effective connectivity in major depression: a study with fMRI and dynamic causal modeling. *Neuroimage*, *43*, 645–55.

Sharot, T., Riccardi, A.M., Raio, C.M., Phelps, E.A. (2007). Neural mechanisms mediating optimism bias. *Nature*, *450*, 102–5.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., et al. (1998). 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. *The Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33; quiz 34–57.

Sheline, Y.I., Barch, D.M., Price, J.L., et al. (2009). The default mode network and self-referential processes in depression. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 1942–7.

Siegle, G.J., Steinhauer, S.R., Thase, M.E., Stenger, V.A., Carter, C.S. (2002). Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biological Psychiatry*, *51*, 693–707.

Stroop, J.R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, *18*, 643–62.

Terhaar, J., Boettger, M.K., Schwier, C., Wagner, G., Israel, A.K., Bar, K.J. (2009). Increased sensitivity to heat pain after sad mood induction in female patients with major depression. *European journal of pain (London, England)*, *14*(5), 559–63.

van der Meer, L., Costafreda, S., Aleman, A., David, A.S. (2010). Self-reflection and the brain: a theoretical review and meta-analysis of neuroimaging studies with implications for schizophrenia. *Neurosci Biobehav Rev*, *34*, 935–46.

Velten, E. Jr. (1968). A laboratory task for induction of mood states. *Behaviour Research and Therapy*, *6*, 473–82.

Verbruggen, F., Liefooghe, B., Vandierendonck, A., Demanet, J. (2007). Short cue presentations encourage advance task preparation: a recipe to diminish the residual switch cost. *Journal of Experimental Psychology. Learning, Memory, and Cognition*, *33*, 342–56.

Wagner, G., Koch, K., Schachtzabel, C., Reichenbach, J.R., Sauer, H., Schlösser, R.G. (2008). Enhanced rostral anterior cingulate cortex activation during cognitive control is related to orbitofrontal volume reduction in unipolar depression. *Journal of Psychiatry & Neuroscience*, *33*, 199–208.

Wagner, G., Koschke, M., Leuf, T., Schlosser, R., Bar, K.J. (2009). Reduced heat pain thresholds after sad-mood induction are associated with changes in thalamic activity. *Neuropsychologia*, *47*, 980–87.

Wagner, G., Sinsel, E., Sobanski, T., et al. (2006). Cortical inefficiency in patients with unipolar depression: an event-related FMRI study with the Stroop task. *Biological Psychiatry*, *59*, 958–65.

Walter, M., Henning, A., Grimm, S., et al. (2009). The relationship between aberrant neuronal activation in the pregenual anterior cingulate, altered glutamatergic metabolism, and anhedonia in major depression. *Archieves of General Psychiatry*, *66*, 478–86.

Yoshimura, S., Okamoto, Y., Onoda, K., et al. (2010). Rostral anterior cingulate cortex activity mediates the relationship between the depressive symptoms and the medial prefrontal cortex activity. *Journal of Affective Disorders*, *122*, 76–85.