Journal of Affective Disorders xxx (2018) xxx-xxx

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com

Research paper

Short-term escitalopram treatment normalizes aberrant self-referential processing in major depressive disorder

Emma Komulainen[a], Roope Heikkil [a], Lauri Nummenmaa[b], Tuukka T. Raij[a, c, d], Catherine J. Harmer[e], Erkki Isomets [a], Jesper Ekelund[a, f]

[a] University of Helsinki and Helsinki University Hospital, Psychiatry, Helsinki, Finland
[b] Turku PET Centre and Department of Psychology, University of Turku, Finland
[c] Aalto University, School of Science, Department of Neuroscience and Biomedical Engineering, Espoo, Finland
[d] Aalto NeuroImaging, Aalto University, Espoo, Finland
[e] University Department of Psychiatry, Oxford, UK
[f] Vaasa Hospital District, Department of Psychiatry, Vaasa, Finland

ARTICLE INFO

Keywords:
Depression
Antidepressant
Escitalopram
fMRI
Emotion
Self-referential processing

ABSTRACT

Background: Increased self-focus and negative self-concept play an important role in depression. Antidepressants influence self-referential processing in healthy volunteers, but their function in self-processing of depressed patients remains unknown.
Methods: Thirty-two depressed patients were randomly allocated to receive either escitalopram 10 mg or placebo for one week. After one week, neural responses to positive and negative self-referential adjectives and neutral control stimuli were assessed with functional magnetic resonance imaging. A group of matched healthy volunteers served as a control group.
Results: Escitalopram decreased responses of medial fronto-parietal regions to self-referential words relative to non-emotional control stimuli, driven by increased responses to the control condition. Escitalopram also increased responses in the pre-defined region of the medial prefrontal cortex (MPFC) and the anterior cingulate cortex (ACC) to positive relative to negative words. Importantly, the changes in neural responses occurred before any effect on depressive symptoms, implying a direct effect of escitalopram. Furthermore, the placebo group had decreased responses of the MPFC and the ACC to positive self-referential processing relative to the matched healthy controls. However, neural responses of the escitalopram group and the healthy unmedicated controls were similar.
Limitations: Differences between the groups in self-reported depression symptoms and personality traits may have influenced the results.
Conclusion: One-week treatment with escitalopram normalized aberrant self-referential processing in depressed patients, shifting the focus from the self to the external environment and potentiating positive self-referential processing. This may be an important factor in mechanism of action of antidepressants.

1. Introduction

Cognitive models of depression emphasize the role of biased information processing, manifesting as negative thoughts about self, the world, and the future, in the development and maintenance of depression (Disner et al., 2011). In addition, depression is associated with increased rumination and self-processing and an increased difficulty in focusing on the external environment (Northoff, 2007). This self-focus typically involves negative emotions, resulting in guilt, self-blame, and low self-esteem (Northoff, 2007). Low mood and increased self-focus seem to be interconnected: increased self-focus is associated with increased levels of negative affect (Mor and Winquist, 2002), and negative affect can trigger increased self-referential processing (Clasen et al., 2015), which predicts future depressive symptoms (Clasen et al., 2015; Disner et al., 2016). Accordingly, a recent meta-analysis about biased cognition and depression established that nega-

Corresponding author at: Department of Psychiatry, University of Helsinki, P.O. Box 590, FI-00029 HUS, Finland.
Email address: emma.komulainen@helsinki.fi (E. Komulainen)

https://doi.org/10.1016/j.jad.2018.04.096
Received 13 November 2017; Received in revised form 25 March 2018; Accepted 8 April 2018
Available online xxx
0165-0327/

tive self-referential cognitions predicted both current and future depression, negative self-beliefs being the strongest predictor (Phillips et al., 2010).

Cognitive psychotherapy targets biased information processing and the underlying maladaptive cognitive schemas in depression (Beck and Alford, 2014), but antidepressants also directly influence processing of emotional information (Harmer and Cowen, 2013). They potentiate positive information processing, such as recognition of happy facial expressions, at an early stage of treatment before any effect on depressive symptoms or subjective feelings is apparent (Harmer et al., 2003a; Harmer et al., 2009). Moreover, this change predicts future treatment response (Shiroma et al., 2014; Tranter et al., 2009), suggesting altered emotional processing as a key mechanism of antidepressant action, compatible with the cognitive models of depression.

Self-referential processing activates the cortical midline structures (CMS) of the brain, including the medial prefrontal cortex (MPFC), anterior and posterior cingulate cortex (ACC and PCC), and the precuneus (Northoff et al., 2006; van der Meer et al., 2010). MPFC and ACC are densely interconnected with limbic and subcortical structures as well as lateral cortical regions (Northoff et al., 2006; Sheline et al., 2010) and have a central role in the neural networks impaired in depression (Drevets et al., 2008; Kaiser et al., 2015). Accordingly, in depression self-focus seems to be particularly associated with elevated activation of the MPFC and the ACC (Lemogne et al., 2012; Lemogne et al., 2009). Antidepressants also influence these regions during rest and various emotional tasks (Ma, 2015; Wang et al., 2015). Moreover, neural responses in the MPFC and the ACC appear to be a promising biomarker to predict response to antidepressants (Phillips et al., 2015), further highlighting the importance of this brain region in antidepressant action.

Previous studies have shown that several antidepressants rapidly modulate self-referential processing in healthy volunteers. A single dose of mirtazapine (SNRAn, serotonin and norepinephrine receptor antagonist) decreased responses of the MPFC and the ACC (Komulainen et al., 2016), and a three-week administration of escitalopram (SRI, serotonin reuptake inhibitor) decreased responses of the PCC/precuneus (Matthews et al., 2010) to emotional words processed in a self-referential manner. However, the function of antidepressants in self-referential processing in the CMS of depressed patients remains unknown.

In the current study, we therefore assessed how a one-week administration of escitalopram modulates neural responses to self-referential information in treatment-seeking individuals with major depressive disorder. We hypothesized that (1) escitalopram would modulate function of the CMS, particularly in the MPFC and the ACC. Considering the negative information processing bias associated with depression and the fact that antidepressants have the potency to correct this bias, we also hypothesized that (2) escitalopram would decrease responses to negative and increase responses to positive self-referential stimuli.

2. Methods

2.1. Subjects and study design

The study was investigator-initiated, double-blind, randomized, and placebo-controlled. Altogether 37 treatment-seeking depressed individuals were recruited between June 2012 and April 2015 from the Finnish Student Health Service (an organization providing healthcare services for university students in Finland), units of Helsinki and Espoo. All subjects were screened with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (Vreeburg et al., 2009) by an investigator (either psychiatrist or psychiatric resident) of the research group to meet the DSM-IV criteria for major depressive disorder (MDD). Depression had to be unipolar and the current episode either of mild or moderate severity (Montgomery- sberg Rating Scale (MADRS) (Montgomery and Asberg, 1979) score 15 30). Subjects were native Finnish-speaking adults (18 65 years) with no current antidepressant medication (for minimum of four months prior to the study) or psychotherapy.

Other exclusion criteria were psychotic disorder, borderline, schizotypal or schizoid personality disorder, primary anxiety disorder (evaluated as clinically primary to MDD by the interviewer), significant suicidal ideation or previous suicide attempt, severe unstable somatic illness, depression due to somatic illness or substance use, life-time alcohol or drug dependence, alcohol or drug abuse during the last 12 months, current use of illicit drugs (cannabis during last three months, other drugs during last month), excessive consumption of alcohol (>24 U/week for men and >16 U/week for women), current use of an antipsychotic agent, mood stabilizer, systemic corticosteroid, beta blockers, or benzodiazepine, or a contraindication for magnetic resonance imaging (MRI).

The study protocol was approved by the Ethics Committee of Helsinki and Uusimaa Hospital District. Written informed consent was obtained from each participant.

Subjects were randomly allocated into two groups to receive either placebo or escitalopram 10 mg once per day in the morning for one week ($+/-$ one day was allowed), after which functional magnetic resonance imaging (fMRI) was performed. Three subjects dropped out of the study during the first week, one subject could not undergo MRI due to excessive anxiety, and one subject's MRI failed due to technical issues. Thus, 32 subjects ($n = 17$ for escitalopram group, $n = 15$ for placebo group) comprised the final study sample. Comorbid anxiety disorders were detected in the SCID for 17 patients, with no significant differences between the groups (Table S1).

To investigate the effect of depression (rather than antidepressant effect) on self-referential processing, we performed further analyses using a matched group of healthy, unmedicated volunteers ($n = 15$) from our previous study (Komulainen et al., 2016) as controls. There were no significant differences in age ($p = 0.258$ in independent samples t-test), gender ($p = 0.427$ in Fisher's exact test), or education level (both groups consisted of university students) between the groups (healthy controls and placebo group of the depressed patients).

2.2. Symptoms and subjective ratings

Before randomization (day 0) and again on the measurement day (day 7), depression severity was assessed with MADRS (Montgomery and Asberg, 1979) and the subjects completed questionnaires including Beck Depression Inventory (BDI-II (Beck et al., 1961)), Beck Anxiety Inventory (BAI (Beck et al., 1988)), and Perceived Social Support Scale Revised (PSSS-R (Blumenthal et al., 1987)). A questionnaire of current affective states was repeated daily (day 0 day 7). The questionnaire consisted of words adapted from Russel's circumplex model of affect (Russell, 1980) and was answered on a five-point Likert scale. The words formed a circumplex with two dimensions, valence and arousal (see (Komulainen et al., 2016) for details). The Five Factor personality traits were measured at baseline using a Finnish version of NEO Five Factor Inventory (NEO-FFI) (Costa and McCrae, 1992). On the measurement day the subjects were also asked about possible side effects of the medication and to guess whether they had been given escitalopram or placebo.

2.3. Task and stimuli for fMRI

During fMRI the participants were briefly (for one second) shown a total of 80 words: 60 adjectives describing 30 unequivocally positive (e.g. honest, reliable, sympathetic) and 30 unequivocally negative (e.g. irresponsible, selfish, lazy) personality features in Finnish and 20 neutral words (10 times left [vasen] and 10 times right [oikea]), in an event-related design with an inter-stimulus interval randomly varying between 5000 ms and 9500 ms. The Presentation software (Neurobehavioral Systems Inc., Albany, CA, USA) controlled the stimulus delivery. Total duration of the task was 11 minutes. The participants were asked to imagine overhearing two people talking about them using the word presented on the screen and to imagine how they would feel. They were asked to categorize the words accordingly as either positive (i.e. they would feel pleasant when being described with the word) or negative (i.e. they would feel unpleasant) as quickly and accurately as possible, using a response key box. As a neutral control task, they were asked to identify the word left or right by pressing the corresponding button.

After fMRI, the participants completed a surprise memory test. In the free recall task, the participants were asked to write down as many words as they could remember from the categorization task. In a recognition memory task, 60 adjectives from the categorization task (targets) and 60 new adjectives (distracters) were shown on a computer screen for one second in a random order. The participants were asked to indicate with key presses as quickly and accurately as possible whether or not each word was presented in the categorization task. The distracters were matched with the target words by length, frequency, and imageability (Komulainen et al., 2016).

2.4. Statistical analysis of baseline characteristics and subjective ratings

Statistical analyses of baseline characteristics, questionnaires, and behavioral data were performed with SPSS Statistics software, version 21 (IBM Corporation, Armonk, NY, USA). Independent samples t-test was used to analyze comprehensive school grade-point average. A non-parametric Mann-Whitney U-test was used to analyze age, duration of current depression episode, and number of previous episodes due to their skewed distributions in the study sample. A mixed model analysis of variance (ANOVA) with measurement time and group as factors was used to analyze mood and anxiety ratings. The significant main effects and interactions were further broken down with post hoc comparisons using Bonferroni correction. For the affective state questionnaire, each sector of the circumplex model was analyzed separately (negative affect (NA), positive affect (PA), negative affect with high arousal (NA-HA), positive affect with high arousal (PA-HA), negative affect with low arousal (NA-LA), positive affect with low arousal (PA-LA), high arousal (HA), and low arousal (LA)).

2.5. Statistical analysis of behavioral data

Repeated-measures ANOVAs (group valence) were used to compare the reaction times between the groups in the emotional categorization task and to compare the number of correct words in the free recall test. Greenhouse-Geisser correction was used where assumption of sphericity was not met. The significant effects were analyzed further with appropriate post hoc comparisons using Bonferoni correction. For the word recognition task, the non-parametric discrimination index A (Grier, 1971) was calculated using a formula: $A = 0.5 + [(H\ FA)(1 + H\ FA)]/[(4H(1\ FA)]$, where H = hits/targets and FA = false alarms/distractors. A repeated-measures ANOVA (group valence) was conducted to compare the performance of the groups.

2.6. fMRI acquisition and analysis

The MR imaging was performed on a 3 T MAGNETOM Skyra whole-body scanner (Siemens Healthcare, Erlangen, Germany) at the Advanced Magnetic Imaging Center, Aalto NeuroImaging, Aalto University School of Science. Image acquisition and analysis in detail have been described previously (Komulainen et al., 2016). Preprocessing and analysis were performed with SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/). EPI images were corrected for slice acquisition time, realigned to the first scan by rigid-body transformation, and co-registered to the individual's anatomical MRI. Anatomical MRI was normalized to standard template (Montreal Neuroimaging Institute template) with SPM's unified segmentation/normalization -algorithm (Ashburner and Friston, 2005), and the resulting deformation field was applied to the EPI images. The EPI images were finally smoothed with Gaussian kernel of FWHM 8 mm.

Five individual first-level contrast images were created: positive and negative > neutral words, positive > neutral words, negative > neutral words, positive > negative words and negative > positive words. At the second level, the first-level contrast images were used in a new general linear model to estimate population-level effects. The group differences, i.e. the effect of escitalopram (drug group versus placebo group) and the effect of depression (placebo group versus healthy volunteers), were assessed with independent samples t-test. In the whole-brain analysis the statistical threshold was set at $p < 0.05$, FDR corrected at cluster level (primary uncorrected voxel-wise threshold at $p < 0.01$). We performed a region of interest (ROI) analysis with an a priori ROI in the MPFC and ACC. The ROI was created with WFU PickAtlas software (Maldjian et al., 2003), using anatomical masks of the MPFC (medial superior frontal gyrus) combined with the ACC from the AAL atlas (Tzourio-Mazoyer et al., 2002). ROI analyses and percentage signal change were computed with MarsBaR software (http://marsbar.sourceforge.net/).

3. Results

3.1. Baseline characteristics and questionnaires

There were no significant differences between the groups in gender (8/17 male in drug group, 6/15 male in placebo group), comprehensive school grade-point average, duration of current depressive episode, or number of previous episodes, but the drug group was older than the placebo group. The placebo group scored higher on the personality trait neuroticism and lower on agreeableness than the drug group (Table 1). We found a significant effect of time in MADRS scores, but no significant main effect of group or group*time interaction. There was a significant main effect of group and time in BDI scores, placebo group having higher scores at both time points, but no significant group*time interaction (Table 2).

For the daily affective states (Figure S1), there was a significant main effect of time ($F(7,28.064) = 4.34$, $p = 0.002$) and group*time interaction ($F(7,28.064) = 3.42$, $p = 0.009$) for NA. In the post hoc comparisons, the only significant differences were in the drug group between day 0 and day 3 (mean NA 5.64 at day 0 and 4.07 at day 3, $p = 0.043$) and between day 3 and day 5 (mean NA 5.94 at day 5, $p = 0.010$). There was also a significant group*time interaction in the ANOVA of PA ($F(7,26.204) = 4.28$, $p = 0.003$), PA-HA ($F(7,26.500) = 4.40$, $p = 0.002$), and HA ($F(7,26.713) = 2.53$, $p = 0.039$), but no post hoc comparison survived multiple comparison correction.

Table 1
Baseline characteristics.

	Drug group	Placebo group		
	Mean (SD)	Mean (SD)	t	p
Neuroticism	28.8 (7.75)	34.6 (4.62)	−2.43	0.022
Agreeableness	34.1 (4.63)	29.3 (7.22)	2.22	0.035
Conscientiousness	23.1 (6.02)	20.5 (6.30)	1.17	0.251
Openness	28.9 (6.66)	30.7 (5.77)	−0.77	0.447
Extraversion	18.6 (6.83)	21.1 (9.44)	−0.83	0.414
Grade-point average (4 10)	8.7 (0.65)	8.9 (0.56)	−0.79	0.439

	Drug group	Placebo group	
	Median	Median	P[a]
Number of previous MDEs	1	1	0.888
Duration of this MDE (weeks)	31	43	0.331
Age	27	23	0.046

Grade-point average = grade-point average of comprehensive school, MDE = major depression episode.
[a] Mann-Whitney U-test.

28 subjects (88%) guessed correctly whether they were having escitalopram or placebo. 14 subjects (82%) from the escitalopram group and 4 subjects (27%) from the placebo group reported side effects. The most common side effects were nausea or other gastrointestinal symptoms.

3.2. Behavioral results

One subject from the drug group was excluded from the analysis of reaction times as an outlier (median reaction time > 3SD from the group mean). One subject from the drug group was excluded from the free recall memory test due to missing data. For reaction times on the emotional categorization task (Table S2), a main effect of valence ($F(1.373,57.656) = 11.56$, $= 0.686$, $p < 0.001$) and a group*valence interaction ($F(2.746,57.656) = 3.37$, $= 0.686$, $p = 0.028$) were found. Three subsequent one-way ANOVAs showed that group difference was present for positive ($F(2,42) = 3.71$, $p = 0.033$), but not for negative ($F(2,42) = 1.89$, $p = 0.163$) or neutral ($F(2,42) = 1.16$, $p = 0.325$) words. In post hoc tests using Bonferroni adjusted alpha-levels of 0.017, the drug group performed significantly faster in categorization of positive words than the placebo group ($t(29) = 2.75$, $p = 0.029$), but no difference was found between drug group and controls ($t(28) = 1.30$, $p = 0.204$) or placebo group and controls ($t(27) = 1.34$, $p = 0.190$). No differences between the groups were found in either free recall task or recognition accuracy.

3.3. fMRI results

Difference between escitalopram and placebo group: ROI analysis. In the ROI analysis of the MPFC/ACC (Fig. 1A), the escitalopram group had significantly increased responses to positive > negative words relative to the placebo group ($p = 0.033$). Signal changes (%) in this ROI (Fig. 1B) revealed increased responses to positive words in the escitalopram group. No significant differences between the groups were found in any other contrast.

Difference between depressed patients and healthy controls: ROI analysis. In the MPFC/ACC depressed patients (placebo group) had lower responses than healthy controls in the contrast of positive > negative words ($p = 0.012$). Signal changes (%) showed that depressed patients receiving placebo had lower responses to positive relative to negative self-referential words, whereas healthy controls had similar responses to both positive and negative words (Fig. 1B). There were no significant group differences in any other contrast.

Difference between escitalopram and placebo group: whole-brain analysis. In the whole-brain analysis, the escitalopram group had decreased responses to self-referential processing (positive and negative > neutral words) in two posterior clusters located in the medial parietal and frontal cortex compared with the placebo group (Fig. 2A). The parietal cluster includes the anterior precuneus, somatosensory cortex, superior parietal cortex (SPC), and the right angular gyrus. The frontal cluster includes the middle cingulate cortex (MCC), primary motor cortices, supplementary motor area (SMA), and the precentral sulcus (corresponding to the frontal eye field (FEF) region (Fox et al., 2005)) (Table 3). Percentage signal changes from the significant clusters revealed that the escitalopram group had markedly higher responses to neutral control words (Fig. 2B and C).

Since clusters of this contrast (positive and negative > neutral words) by visual inspection span fronto-parietal regions implicated in both the default mode network (DMN) and the attention system, we performed further analysis aiming to clarify the brain network in which the effect of escitalopram is most prominently seen. We performed a ROI analysis based on automated meta-analyses of (1) the default mode network and (2) the attention network generated by Neurosynth (Yarkoni et al., 2011) (www.neurosynth.org, see details in Tables S3, S4, S5 and Fig. S2). Most of the ROI analyses with clusters from both networks resulted in a significant group difference with decreased responses in the escitalopram group. Only in the MPFC/ACC and the PCC from the DMN and the MCC from the attention network no significant group difference was found (in the hippocampus only a trend-level group difference, see Table S6).

The escitalopram group also had lower responses to positive self-referential processing (positive > neutral words) than the placebo group in similar regions as above, additionally spanning the right posterior thalamus (Fig. 3, Table 3). No significant group differences emerged in the contrasts of negative > neutral words, positive > negative words, or negative > positive words in the whole-brain analysis. However, there was a trend towards decreased responses to negative self-referential processing (negative > neutral words) in the escitalopram group in the cluster with the peak voxel in the precuneus (uncorrected $p = 0.017$).

Given the difference between the groups in baseline BDI and neuroticism scores, we further tested if adding the baseline BDI or neuroticism score as a covariate in each second-level model influences the results. It did not weaken the results in the contrast of positive and negative > neutral words or positive > neutral words. In the contrast of positive > negative words, the ROI analysis did not reach statistical significance when controlling for BDI ($p = 0.408$) or neuroticism ($p = 0.066$). However, no significant correlation emerged between the BDI or neuroticism score and the mean signal extracted from the ROI for positive or negative words in either of the groups ($p > 0.19$ in all correlation analyses).

Difference between depressed patients and healthy controls: whole-brain analysis. In the whole-brain analysis, depressed patients (placebo group) had lower responses of the DMPFC and the perigenual ACC (peak voxel at the right DMPFC; MNI coordinates 6, 38, 46) to positive relative to negative self-referential processing (positive > neg-

Table 2
Mixed model analysis of variance (ANOVA) of the questionnaires.

		Drug group	Placebo group	Main effect of week		Main effect of group		Interaction	
		Mean (SD)	Mean (SD)	F	P	F	P	F	P
MADRS	week 0	22.1 (3.91)	24.5 (4.05)	F(1,30) = 15.08	0.001	F(1,30) = 2.39	0.133	F(1,30) = 0.09	0.765
	week1	20.2 (4.56)	22.3 (4.95)						
BDI	week 0	22.7 (5.79)	31.3 (6.73)	F(1,27.414) = 15.7	0.001	F(1,29.288) = 13.18	0.001	F(1,27.414) = 0.87)	0.360
	week1	20.5 (5.22)	27.4 (7.92)						
BAI	week 0	13.5 (9.31)	19.5 (9.72)	F(1,27.798) = 0.15	0.697	F(1,29.256) = 2.06	0.162	F(1,27.798) = 3.49	0.072
	week1	14.5 (9.46)	17.9 (8.76)						
PSSS-R	week 0	47.3 (12.49)	41.1 (7.54)	F(1,27.739) = 0.04	0.845	F(1,29.043) = 2.46	0.128	F(1,27.739) = 0.83	0.370
	week1	45.9 (10.71)	41.9 (8.08)						

MADRS = Montgomery- sberg Depression Rating Scale, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory, PSSS-R = Perceived Social Support Scale Revised.

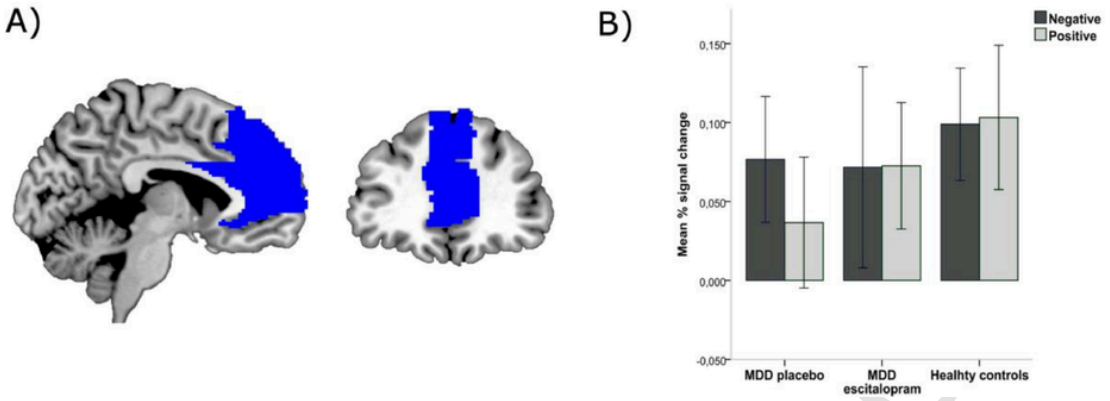

Fig. 1. (A) The anatomical mask of the medial prefrontal cortex and anterior cingulum (from AAL Atlas) that was used as an a priori ROI. (B) Percentage signal change extracted from the ROI for positive and negative adjectives in the placebo and escitalopram groups as well as in healthy volunteers. Error bars represent standard error of mean.

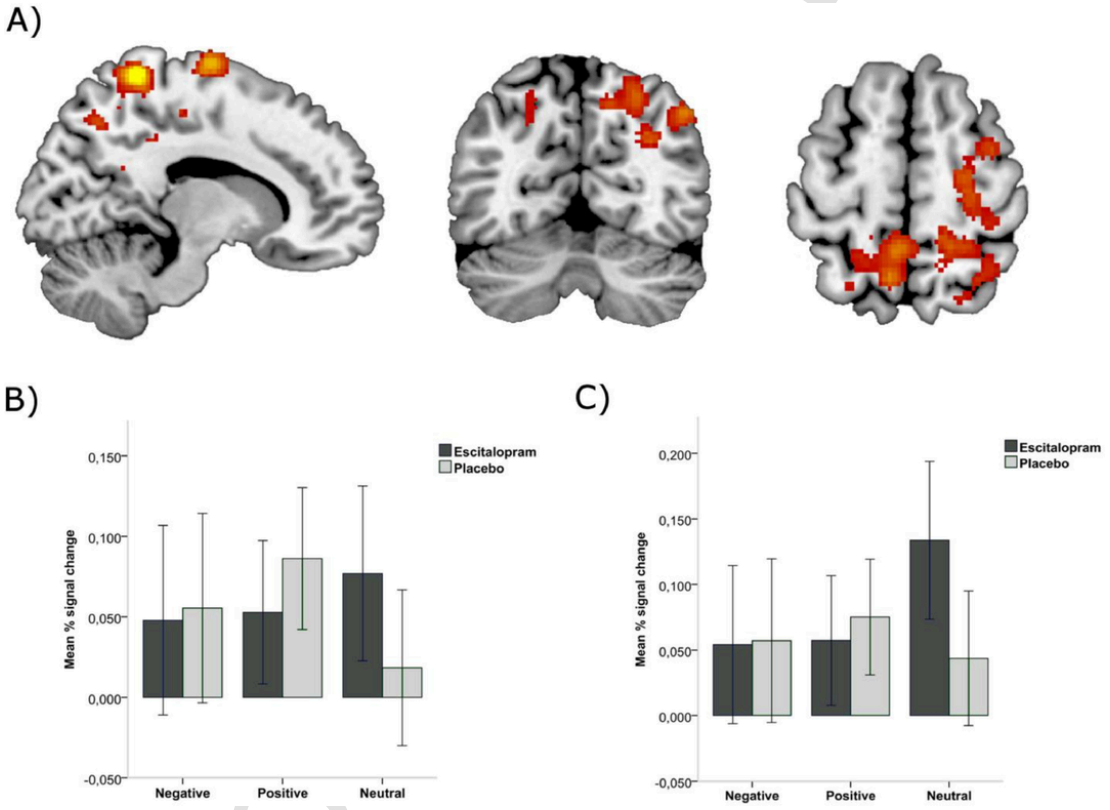

Fig. 2. (A) Regions with decreased responses to self-referential words relative to neutral words in the escitalopram group compared with the placebo group ($p < 0.05$, FDR-corrected at cluster level). x = 12, y = −65, z = 60. Percentage signal change for each stimulus type extracted from (B) the anterior cluster (including middle cingulate cortex, primary motor cortices, supplementary motor area, and the medial end of the precentral sulcus) and (C) the posterior cluster (including precuneus, somatosensory cortex, superior parietal cortex, and right angular gyrus). Error bars represent standard error of mean.

ative words) compared with healthy controls (FDR-corrected $p = 0.073$, FWE-corrected $p = 0.046$, Fig. 4). Comparing the escitalopram group with healthy volunteers revealed no significant group differences. There were no significant group differences in any other contrast.

4. Discussion

4.1. Effect of escitalopram on self-referential processing

In this placebo-controlled, double-blind study, we assessed neural responses to self-referential processing in treatment-seeking depressed patients after a one-week treatment with escitalopram. Escitalopram decreased neural responses to self-referential processing in the posterior medial cortex, including the precuneus. The effect was mostly driven by increased responses to the neutral control condition. In accordance with our primary hypothesis, escitalopram also increased responses to positive compared with negative self-referential words in the MPFC and ACC, driven by increased responses to positive words. Importantly, the changes occurred before any effect of the antidepressant on depressive symptoms was achieved, as there were no group differences in improvement of depressive symptoms. Yet the neural responses in the drug group were already compatible with those in healthy unmedicated volunteers. This implies that the normalization of aberrant neural responses is not confounded by improvement of mood,

Table 3
Peak activations of the clusters showing significantly decreased activation in the escitalopram group compared with the placebo group ($p < 0.05$, FDR-corrected at cluster level). SFG = superior frontal gyrus, PostCG = postcentral gyrus. Coordinates in MNI space.

Contrast	Region	P-value	Z-value	Coordinates
Self-referential > neutral	Precuneus	0.001	4.63	12, −46, 70
	Right SFG	0.010	3.60	14, −10, 76
Positive > neutral	Right PostCG	0.001	4.59	14, −44, 70

but precedes the therapeutic effect of escitalopram, thus being a plausible factor in its mechanisms of effect.

Depression is associated with functional abnormalities in both the DMN and the attention network. Diminished ability to deactivate the DMN appropriately during emotional tasks (Sheline et al., 2009), and dominance of the DMN over the attention network (Kaiser et al., 2015), linked to maladaptive rumination (Hamilton et al., 2011), has been observed in depressed patients. This imbalance of brain networks may be behind the biased attention towards internal thoughts and sensations at the expense of decreased attention to external environment and current cognitive tasks (Kaiser et al., 2015). We found escitalopram to modulate self-referential processing in a cluster that peaked in the anterior precuneus. The precuneus is part of the DMN, known to activate during rest and deactivate during cognitive tasks (Fox et al., 2005). The DMN has been implicated in internally focused tasks, such as stimulus-independent thoughts (mind wandering) and retrieval of autobiographic memories, but also in attending to the external environment, particularly in passive, cognitively low-demanding conditions (so-called passive watchfulness) (Buckner et al., 2008; Greicius et al., 2004). The clusters where we found escitalopram to modulate self-referential processing included the regions implicated in external monitoring (precuneus, SPC, and somatosensory cortex (Andrews-Hanna et al., 2010; Davey et al., 2016)), but also parietal and frontal regions of the dorsal attention network such as the SPC, SMA, and FEF (Corbetta et al., 2002; Vincent et al., 2008). The complementary ROI analysis using the automated meta-analysis of the attention network and the DMN as ROIs further suggested that escitalopram in fact modulated the activity of both networks, and particularly in the regions of the DMN not included in the core-self regions (such as MPFC and PCC (Davey et al., 2016)). Increased responses to neutral control condition (responding left or right) in these regions may be interpreted as an improved ability to shift attention from internal to external milieu.

In the same region we also observed decreased responses to positive > neutral words in the escitalopram group, whereas decreased responses to negative > neutral words were only found at trend-level. Thus, even though the effect mostly derived from increased responses to neutral words it was, unlike expected, stronger for positive > neutral words than negative > neutral words. However, previous findings concerning the role of valence are not unequivocal, one study finding specific effect of citalopram on negative self-referential processing (Di Simplicio et al., 2012), another study reporting decreased brain responses to positive relative to negative self-referential words after reboxetine treatment (Norbury et al., 2008) and another study finding the effect of agomelatine to be unspecific to valence (Delaveau et al., 2016). Several factors, such as type and dose of antidepressant, dura-

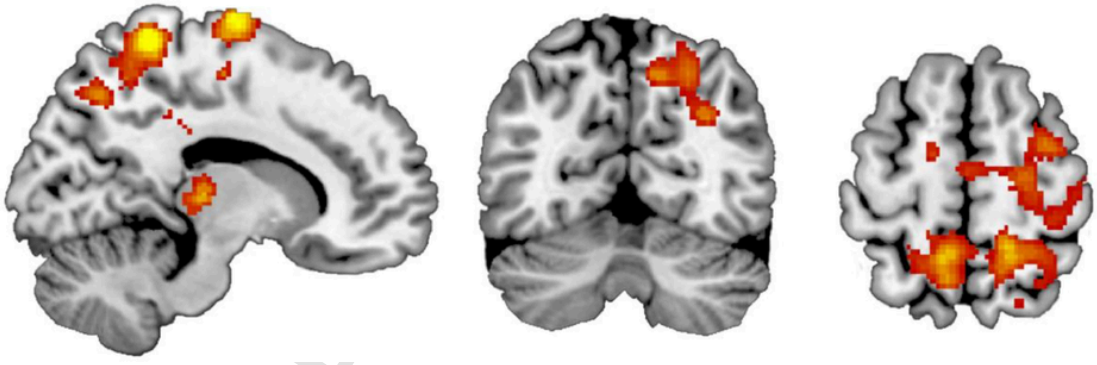

Fig. 3. Regions with decreased responses to positive words relative to neutral words in the escitalopram group compared with the placebo group ($p < 0.05$, FDR-corrected at cluster level). x = 12, y = −65, z = 60.

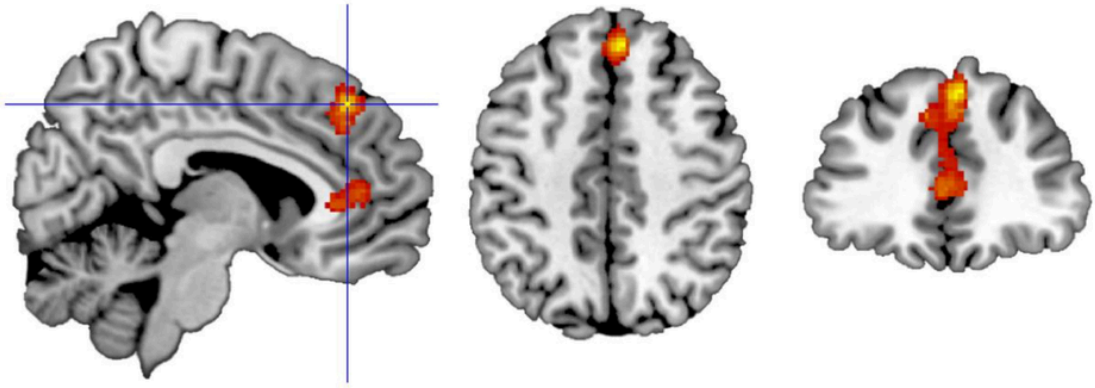

Fig. 4. Regions with decreased responses to positive relative to negative self-referential words in depressed patients (placebo group) compared with healthy controls ($p < 0.05$, FDR-corrected at cluster level). The crosshair is at the peak voxel of the cluster (MNI coordinates 6, 38, 46).

tion of treatment and the study sample (healthy subjects vs patients, subtype of depression) may influence the results, and more studies especially in depressed patients are needed to shed light on this question.

Consistent with our primary hypothesis, we observed a valence-specific change in neural responses to self-referential processing in the MPFC and ACC. Escitalopram was found to improve positive over negative self-referential processing. This was supported at the behavioral level by shorter reaction times to positive words, thought to be a marker of increased attention to positive stimuli with treatment (Harmer et al., 2004). The MPFC and ACC have a role in self-referential processing in the emotional domain; tracking, evaluating, and reappraising self-relatedness of interoceptive and exteroceptive information (Northoff et al., 2006). The MPFC, often together with the ACC, is implicated in increased self-focus of depressed patients (Lemogne et al., 2012; Lemogne et al., 2009) and has been suggested as a core region of conscious self-awareness (Davey et al., 2016). Our result suggests that escitalopram may alter self-referential processing in the MPFC/ACC by modulating the evaluation of self-referential events and tagging of emotional content to them. This change in the core-self system may ultimately lead to modulation of the conscious experience of self, normalizing the negatively biased self-perception. As the change in neural responses occurred before any change in depressive symptoms or subjective feelings was observed, it is possible that the translation of these early effects to the subjective, conscious level of emotional experiences requires time and re-learning of emotional associations in the real-life environment (Harmer and Cowen, 2013).

We found no effect of escitalopram on emotional memory. Previous studies, although not all (Browning et al., 2007), have found serotonergic and noradrenergic antidepressants to increase memory of positive self-referential words in healthy volunteers (Harmer et al., 2003b; Harmer et al., 2004). However, there are no previous studies of depressed patients treated with an SRI antidepressant. Further, in our study the emotional memory task was performed on average 90 min after the categorization task whereas previous studies with healthy volunteers have performed memory task immediately or 15 min after categorization task, which may influence the result. Also, it is possible that the effect is not strong enough to show at a behavior level despite core differences in neural response.

4.2. Effect of depression on self-referential processing

When comparing the depressed patients of the placebo group to healthy volunteers matching in age and gender, depressed patients had attenuated responses to positive relative to negative self-referential processing in the DMPFC and the perigenual ACC. This is compatible with negatively biased information processing associated with depression (Disner et al., 2011) and replicates the previously found effects of depression on self-referential processing in the anterior CMS (Grimm et al., 2009; Lemogne et al., 2009; Wagner et al., 2013; Yoshimura et al., 2010). Importantly, when comparing the escitalopram group of the depressed patients with healthy subjects, no significant difference was found, suggesting that escitalopram indeed normalizes the negative bias in self-referential processing. The valence-specific effect of depression on self-referential processing was found in precisely the same regions as in previous studies, spanning the DMPFC and the perigenual ACC (Johnson et al., 2009; Lemogne et al., 2009; Wagner et al., 2013; Yoshimura et al., 2010). Particularly the dorsal part of the MPFC has been shown to uniquely activate in self-referential processing of depressed patients (with a peak activation at 9, 42, 33 in MNI space, near to the peak activation in our study) (Lemogne et al., 2009), and may be a key region behind the negatively biased self-focus typical of depression, as well as a key target for treatment interventions.

5. Limitations

The study sample included only university students and mostly young adults. This may hinder the generalization of the results to older and less educated populations. However, the clinical characteristics of the study subjects, such as median duration of the current depression episode of more than six months and half of the patients having a comorbid anxiety disorder, as well as the important fact that all subjects were treatment-seeking patients, do support the study sample resembling the depressed patients physicians encounter in their everyday practice, strengthening the clinical significance of the results.

The difference in BDI and neuroticism scores between the groups can be argued to influence the results. However, the lack of a significant correlation between BDI or neuroticism and the extracted signal in either of the groups suggests that the effect was not driven by these differences. Also, there were no significant group differences in an interview-based MADRS, which further supports that the difference between the escitalopram group and the placebo group in neural responses did not derive from a difference in depression severity.

It should be further noted that the study design is cross-sectional and does not allow inferring if the activation is decreased/increased by escitalopram. Thus, when decrease or increase in activation is reported, it actually means higher or lower activation compared with the placebo group which is interpreted as a change in activation by escitalopram.

6. Conclusions

Escitalopram decreased responses of the posterior medial regions centered in the precuneus to self-referential processing relative to the neutral control condition. It also increased responses of the MPFC and the ACC to positive self-referential processing. These changes occurred before any effect of the antidepressant on depressive symptoms was observed, indicating that the changes were caused by the treatment directly, rather than indirectly via improvements in symptomatic state. Escitalopram seems to improve the regulation of self-referential processing, shifting attention from the internal to the external milieu. It also seems to specifically potentiate positive self-processing, possibly leading to normalization of increased and negatively biased self-focus. This finding contributes to the understanding of the mechanism of action of antidepressants.

Conflict of interest

Author CJH has received consultancy fees from Lundbeck, P1vital, Servier. She is a company director and shareholder of Oxford Psychologists Ltd. She holds current grant income from UCB and J&J. All other authors declare that they have no conflicts of interest.

Acknowledgment

We thank Marita Kattelus for help with data acquisition. We also thank Dr. Sini Innilä and other personnel of the Finnish Student Health Service in Helsinki and Espoo for help with patient recruitment.

Funding

This work was supported by the Academy of Finland (grant number 250935 for JE and grant number 294897 for LN).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2018.04.096.

References

Andrews-Hanna, J.R., Reidler, J.S., Huang, C., Buckner, R.L., 2010. Evidence for the Default Network's Role in Spontaneous Cognition. J Neurophysiol. 104, 322.

Ashburner, J., Friston, K.J., 2005. Unified segmentation. NeuroImage 26, 839–851.

Beck, A.T., Alford, B.A., 2014. Depression, Causes and Treatment, second ed. University of Pensylvania Press, Philadelphia, Pensylvania.

Beck, A.T., Epstein, N., Brown, G., Steer, R.A., 1988. An inventory for measuring clinical anxiety: psychometric properties. J. Consul. Clin. Psychol. 56, 893–897.

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.

Blumenthal, J.A., Burg, M.M., Barefoot, J., Williams, R.B., Haney, T., Zimet, G., 1987. Social support, type A behavior, and coronary artery disease. Psychosom. Med. 49, 331–340.

Browning, M., Reid, C., Cowen, P.J., Goodwin, G.M., Harmer, C.J., 2007. A single dose of citalopram increases fear recognition in healthy subjects. J. Psychopharmacol. 21, 684–690.

Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The brain's default network: anatomy, function, and relevance to disease. An. NY Acad. Sci. 1124, 1–38.

Clasen, P.C., Fisher, A.J., Beevers, C.G., 2015. Mood-reactive self-esteem and depression vulnerability: person-specific symptom dynamics via smart phone assessment. PLoS one 10, e0129774.

Corbetta, M., Kincade, J.M., Shulman, G.L., 2002. Neural systems for visual orienting and their relationships to spatial working memory. J. Cognitive Neurosci. 14, 508–523.

Costa, P.J., McCrae, R., 1992. NEO-PI-R Professional Manual. Psychological Assessment Resources, Inc., Odessa, FL.

Davey, C.G., Pujol, J., Harrison, B.J., 2016. Mapping the self in the brain's default mode network. NeuroImage 132, 390–397.

Delaveau, P., Jabourian, M., Lemogne, C., Alla li, N., Choucha, W., Girault, N., Lehericy, S., Laredo, J., Fossati, P., 2016. Antidepressant short-term and long-term brain effects during self-referential processing in major depression. Psychiatry Res. 247, 17–24.

Di Simplicio, M., Norbury, R., Harmer, C.J., 2012. Short-term antidepressant administration reduces negative self-referential processing in the medial prefrontal cortex in subjects at risk for depression. Mol Psychiatry 17, 503–510.

Disner, S.G., Beevers, C.G., Haigh, E.A., Beck, A.T., 2011. Neural mechanisms of the cognitive model of depression. Nat. Rev. Neurosci. 12, 467–477.

Disner, S.G., Shumake, J.D., Beevers, C.G., 2016. Self-referential schemas and attentional bias predict severity and naturalistic course of depression symptoms. Cogn. Emot. 1–13.

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 Struct. Fun. 213, 93–118.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci USA 102, 9673–9678.

Greicius, M.D., Srivastava, G., Reiss, A.L., Menon, V., 2004. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc. Natl. Acad. Sci. USA 101, 4637–4642.

Grier J.B., Nonparametric indexes for sensitivity and bias: computing formulas. Psychol Bull 75, 1971, 424-429.Grimm, S., Boesiger, P., Beck, J., Schuepbach, D., Bermpohl, F., Walter, M., Ernst, J., Hell, D., Boeker, H., Northoff, G., 2009. Altered negative BOLD responses in the default-mode network during emotion processing in depressed subjects. Neuropsychopharmacology 34, 932–943.

Hamilton, J.P., Furman, D.J., Chang, C., Thomason, M.E., Dennis, E., Gotlib, I.H., 2011. Default-mode and task-positive network activity in major depressive disorder: implications for adaptive and maladaptive rumination. Biol. Psychiatry 70, 327–333.

Harmer, C.J., Bhagwagar, Z., Perrett, D.I., Vollm, B.A., Cowen, P.J., Goodwin, G.M., 2003. Acute SSRI administration affects the processing of social cues in healthy volunteers. Neuropsychopharmacology 28, 148–152.

Harmer, C.J., Cowen, P.J., 2013. It s the way that you look at it a cognitive neuropsychological account of SSRI action in depression. Phil. Trans. R. Soc B 368, 20120407.

Harmer, C.J., Hill, S.A., Taylor, M.J., Cowen, P.J., Goodwin, G.M., 2003. Toward a neuropsychological theory of antidepressant drug action: increase in positive emotional bias after potentiation of norepinephrine activity. Am. J. Psychiatry 160, 990–992.

Harmer, C.J., O'Sullivan, U., Favaron, E., Massey-Chase, R., Ayres, R., Reinecke, A., Goodwin, G.M., Cowen, P.J., 2009. Effect of acute antidepressant administration on negative affective bias in depressed patients. Am. J. Psychiatry 166, 1178–1184.

Harmer, C.J., Shelley, N.C., Cowen, P.J., Goodwin, G.M., 2004. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. Am. J. Psychiatry 161, 1256–1263.

Johnson, M.K., Nolen-Hoeksema, S., Mitchell, K.J., Levin, Y., 2009. Medial cortex activity, self-reflection and depression. Soc. Cogn. Affect Neurosci. 4, 313–327.

Kaiser, R.H., Andrews-Hanna, J.R., Wager, T.D., Pizzagalli, D.A., 2015. Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting-state functional connectivity. JAMA Psychiatry 72, 603–611.

Komulainen, E., Heikkila, R., Meskanen, K., Raij, T.T., Nummenmaa, L., Lahti, J., Jylha, P., Melartin, T., Harmer, C.J., Isometsa, E., Ekelund, J., 2016. A single dose of mirtazapine attenuates neural responses to self-referential processing. J. Psychopharmacol. 30, 23–32.

Lemogne, C., Delaveau, P., Freton, M., Guionnet, S., Fossati, P., 2012. Medial prefrontal cortex and the self in major depression. J. Affect. Disord. 136, e1–e11.

Lemogne, C., le Bastard, G., Mayberg, H., Volle, E., Bergouignan, L., Lehericy, S., Allilaire, J.F., Fossati, P., 2009. In search of the depressive self: extended medial prefrontal network during self-referential processing in major depression. Soc. Cogn. Affect. Neurosci. 4, 305–312.

Ma, Y., 2015. Neuropsychological mechanism underlying antidepressant effect: a systematic meta-analysis. Mol. Psychiatry 20, 311–319.

Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19, 1233–1239.

Matthews, S.C., Simmons, A.N., Strigo, I.A., Arce, E., Stein, M.B., Paulus, M.P., 2010. Escitalopram attenuates posterior cingulate activity during self-evaluation in healthy volunteers. Psychiatry Res 182, 81–87.

Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 382–389.

Mor, N., Winquist, J., 2002. Self-focused attention and negative affect: a meta-analysis. Psychol Bull. 128, 638–662.

Norbury, R., Mackay, C.E., Cowen, P.J., Goodwin, G.M., Harmer, C.J., 2008. The effects of reboxetine on emotional processing in healthy volunteers: an fMRI study. Mol. Psychiatry. 13, 1011–1020.

Northoff, G., 2007. Psychopathology and pathophysiology of the self in depression - neuropsychiatric hypothesis. J. Affect. Disord. 104, 1–14.

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, 440–457.

Phillips, M.L., Chase, H.W., Sheline, Y.I., Etkin, A., Almeida, J.R., Deckersbach, T., Trivedi, M.H., 2015. Identifying predictors, moderators, and mediators of antidepressant response in major depressive disorder: neuroimaging approaches. Am. J. Psychiatry 172, 124–138.

Phillips, W.J., Hine, D.W., Thorsteinsson, E.B., 2010. Implicit cognition and depression: A meta-analysis. Clin. Psychol. Rev. 30, 691–709.

Russell, J.A., 1980. A circumplex model of affect. J. Pers. Soc. Psychol. 39, 1161–1178.

Sheline, Y.I., Barch, D.M., Price, J.L., Rundle, M.M., Vaishnavi, S.N., Snyder, A.Z., Mintun, M.A., Wang, S., Coalson, R.S., Raichle, M.E., 2009. The default mode network and self-referential processes in depression. Proc. Natl. Acad. Sci. USA 106, 1942–1947.

Sheline, Y.I., Price, J.L., Yan, Z., Mintun, M.A., 2010. Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. Proc. Natl. Acad. Sci. USA 107, 11020–11025.

Shiroma, P.R., Thuras, P., Johns, B., Lim, K.O., 2014. Emotion recognition processing as early predictor of response to 8-week citalopram treatment in late-life depression. Int. J. Geriatr. Psychiatry 29, 1132–1139.

Tranter, R., Bell, D., Gutting, P., Harmer, C., Healy, D., Anderson, I.M., 2009. The effect of serotonergic and noradrenergic antidepressants on face emotion processing in depressed patients. J. Affect. Disord. 118, 87–93.

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

Wagner, G., Koch, K., Schachtzabel, C., Peikert, G., Schultz, C.C., Reichenbach, J.R., Sauer, H., Schlosser, R.G., 2013. Self-referential processing influences functional activation during cognitive control: an fMRI study. Soc. Cogn. Affect. Neurosci. 8, 828–837.

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, 935–946.

Wang, L., Xia, M., Li, K., Zeng, Y., Su, Y., Dai, W., Zhang, Q., Jin, Z., Mitchell, P.B., Yu, X., He, Y., Si, T., 2015. The effects of antidepressant treatment on resting-state functional brain networks in patients with major depressive disorder. Hum. Brain Mapp 36, 768–778.

Vincent, J.L., Kahn, I., Snyder, A.Z., Raichle, M.E., Buckner, R.L., 2008. Evidence for a frontoparietal control system revealed by intrinsic functional connectivity. J. Neurophysiol. 100, 3328.

Vreeburg, S.A., Hoogendijk, W.J., van Pelt, J., Derijk, R.H., Verhagen, J.C., van Dyck, R., Smit, J.H., Zitman, F.G., Penninx, B.W., 2009. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. Arch. Gen. Psychiatry 66, 617–626.

Yarkoni, T., Poldrack, R.A., Nichols, T.E., Van Essen, D.C., Wager, T.D., 2011. Large-scale automated synthesis of human functional neuroimaging data. Nat. Methods 8, 665–670.

Yoshimura, S., Okamoto, Y., Onoda, K., Matsunaga, M., Ueda, K., Suzuki, S.-i., Yamawaki, Shigeto, 2010. Rostral anterior cingulate cortex activity mediates the relationship between the depressive symptoms and the medial prefrontal cortex activity. J. Affect. Disord. 122, 76–85.