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**Subgenual cingulate-amygdala functional disconnection and vulnerability to
melancholic depression**

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ABSTRACT

The syndromic heterogeneity of major depressive disorder (MDD) hinders understanding of the aetiology of predisposing vulnerability traits and underscores the importance of identifying neurobiologically valid phenotypes. Distinctive fMRI biomarkers of vulnerability to MDD subtypes are currently lacking. This study investigated whether remitted melancholic MDD patients, who are at an elevated lifetime risk for depressive episodes, demonstrate distinctive patterns of resting-state connectivity with the subgenual cingulate cortex (SCC), known to be of core pathophysiological importance for severe and familial forms of MDD. We hypothesized that patterns of disrupted SCC connectivity would be a distinguishing feature of melancholia. Sixty-three medication-free remitted MDD (rMDD) patients (33 melancholic, 30 non-melancholic) and 39 never-depressed healthy controls (HC) underwent resting-state fMRI scanning. SCC connectivity was investigated with closely connected bilateral *a priori* regions of interest (ROI) relevant to MDD (anterior temporal, ventromedial prefrontal, dorsomedial prefrontal cortices, amygdala, hippocampus, septal region, and hypothalamus). Decreased (less positive) SCC connectivity with the right parahippocampal gyrus and left amygdala distinguished melancholic rMDD patients from the non-melancholic rMDD and HC groups (cluster-based familywise error-corrected $p \leq 0.007$ over individual *a priori* ROIs corresponding to approximate Bonferroni-corrected $p \leq 0.05$ across all seven *a priori* ROIs). No areas demonstrating increased (more positive) connectivity were observed. Abnormally decreased connectivity of the SCC with the amygdala and parahippocampal gyrus distinguished melancholic from non-melancholic rMDD. These results provide the first resting-state neural signature distinctive of melancholic rMDD and may reflect a subtype-

specific primary vulnerability factor given a lack of association with the number of previous episodes.

INTRODUCTION

Major depressive disorder (MDD) is an inherently heterogeneous condition due to its polythetic diagnostic criteria. A diagnosis of MDD requires a combination of only five of nine possible symptoms, meaning two patients may share only one common symptom. This heterogeneity decreases the likelihood of identifying the underlying neurobiological mechanisms underpinning MDD and underscores the importance of identifying neurobiologically valid depressive phenotypes. The melancholic subtype of MDD, characterized by unvarying low mood and drive as well as psychomotor and vegetative symptoms, has been associated with stable personality features which are present outside the depressed state and increase vulnerability to depression (Hecht *et al*, 1998). Identifying a distinct neural signature for vulnerability to melancholic MDD would provide important evidence of its neurobiological validity and point to an intermediate phenotype (Meyer-Lindenberg and Weinberger, 2006). Neuroimaging studies in patients with current melancholic MDD have provided initial evidence of distinct brain abnormalities including subgenual cingulate cortex (SCC) dysfunction (Pizzagalli *et al*, 2004). However, attempts at identifying markers capable of distinguishing melancholic MDD from other depressive subtypes have been largely unsuccessful. It is furthermore unclear whether the distinctive abnormalities described were correlates of the depressed state rather than vulnerability traits.

Resting-state functional MRI (rsfMRI), which can be used to measure spontaneous low-frequency fluctuations in blood oxygen level-dependent (BOLD) signal in the resting brain (Fox and Raichle, 2007), has become an increasingly popular imaging method for characterizing network-level disruptions associated with psychiatric disorders. There are strong theoretical and practical motivations for acquiring rsfMRI in psychiatric studies: 1) large metabolic demands of the resting brain suggest a critical role in overall brain

functioning (Fox *et al*, 2007), and 2) rsfMRI scans entail short acquisition times and do not require complex cognitive paradigms. The literature describing resting-state network abnormalities in current MDD has grown substantially over the past decade (reviewed in (Dutta *et al*, 2014)). Importantly, a recent rsfMRI study of patients with current MDD revealed decreased effective connectivity in attention and interoception networks in melancholic relative to non-melancholic patients and a healthy control (HC) group (Hyett *et al*, 2015). It is elusive, however, whether these effects reflected differences in symptoms experienced during the depressive episode or neural differences irrespective of current symptom profile. It was further difficult to control for antidepressant effects. Therefore, it remains unknown whether rsfMRI can be used to reveal distinctive signatures of vulnerability traits predisposing to different MDD subtypes.

Studies of brain functioning in MDD highlight the SCC as a key region in the pathophysiology of depression (see (Mayberg, 2003; Ressler and Mayberg, 2007)). In HC participants, SCC BOLD response was associated with guilt proneness (Zahn *et al*, 2009), which is often excessive and overgeneralized in current MDD, particularly in the melancholic subtype. Resting-state glucose metabolism in the SCC has previously been shown to be elevated in current MDD and to normalize upon remission from the depressed state (reviewed in (Ressler *et al*, 2007)). Furthermore, current MDD patients demonstrated greater resting-state connectivity of the SCC with fronto-parieto-limbic regions relative to control participants (Greicius *et al*, 2007; Sheline *et al*, 2010). Green and colleagues (2012) recently demonstrated task-related SCC decoupling in remitted MDD (rMDD) patients during the experience of guilt. Since rMDD patients are at a highly elevated lifetime risk for major depressive episodes (MDE) relative to HC participants (Kupfer, 1991), this suggests guilt-related SCC decoupling may represent a trait vulnerability factor for MDD rather than a correlate of the depressed state. Additionally, although the literature on resting-state

connectivity in depression vulnerability is limited, disrupted SCC connectivity has been observed in young rMDD patients (Gaffrey *et al*, 2012) and in at-risk adolescents (Herringa *et al*, 2013).

The present study employed rsfMRI to investigate functional connectivity of the SCC in fully remitted, medication-free MDD patients with or without a history of melancholic MDEs. By investigating patients with rMDD, the present study is well-suited to identify stable vulnerability traits for experiencing MDEs (Burcusa and Iacono, 2007). The current study used a seed-based analysis approach as this method has been commonly used in rsfMRI studies of current MDD (Dutta *et al*, 2014). We used the SCC seed region identified in Green and colleagues' (2012) study which previously demonstrated decoupling with medial frontal and medial and anterior temporal cortices during the experience of guilt in a rMDD patient group that included a high proportion of melancholic rMDD patients. We hypothesized that melancholic rMDD patients would demonstrate a distinctive pattern of disrupted SCC connectivity when compared with the non-melancholic rMDD patients and HC participants. We further predicted that decreased (less positive) medial prefrontal connectivity with the SCC would distinguish melancholic from non-melancholic rMDD patients. This prediction was based on evidence that the medial prefrontal cortex is necessary for social actions and motivations (Moll *et al*, 2005) which are classically impaired in melancholia (Ebert *et al*, 1995), as well as its known direct anatomical connections to the SCC (Carmichael and Price, 1996; Vogt and Pandya, 1987) and previous work in which it demonstrated guilt-related functional disconnection with the SCC (Green *et al*, 2012).

MATERIALS and METHODS

Participants

The present study was approved by the South Manchester National Health Service Research Ethics Committee. After the study procedures were explained in full, participants gave informed consent (verbal consent for a telephone pre-screening and written consent at the outset of each study visit). Participant recruitment was conducted using online and print advertisements as part of the UK Medical Research Council-funded “Development of Cognitive and Imaging Biomarkers Predicting Risk of Self-Blaming Bias and Recurrence in Major Depression” project (Zahn *et al*, 2015). Participants received compensation for their time and travel expenses. Prior to taking part in the study, 707 volunteers completed a telephone pre-screening to preliminarily assess eligibility (the pre-screening document is available at <http://www.translational-cognitive-neuroscience.org/start/test-materials>). Volunteers who passed the initial screening were then invited to complete a clinical interview overseen by a senior psychiatrist (RZ) at which psychiatric, clinical, and family histories were recorded. The Structured Clinical Interview-I for DSM-IV-TR was used to diagnose past MDEs with or without melancholic features (First *et al*, 2002) with moderate to perfect inter-rater reliability (see Table S1). Exclusion criteria were: current Axis I disorders, history of substance abuse or major medical or neurological disorders, exposure to psychotropic medications within 4 weeks (8 weeks for fluoxetine), and contraindications for MRI scanning. Additionally, the HC group had no history of Axis-I disorders and no first-degree family history of mood disorders or schizophrenia. Following the initial clinical interview, 96 rMDD patients and 48 HC participants were eligible for enrollment into the current study (see Table 1 for a detailed overview of reasons for exclusion). Of these, 63 medication-free patients with rMDD (33 melancholic, 30 non-melancholic) and 39 never-depressed HC participants underwent MRI scanning. One HC participant’s data were excluded due to the presence of a pituitary abnormality.

The rMDD patients (both overall and melancholic) and HC group were closely matched on demographic variables (Table 2), as were the melancholic and non-melancholic rMDD patients (Tables 2–3). The rMDD patients (both overall and melancholic) did not differ from HC group with respect to age, years of education, or sex. Scores on the Beck Depression Inventory (BDI; (Beck *et al*, 1996)) were slightly elevated in the rMDD patients and differed from the HC group ($t(99)=3.71$, $p(\text{unc})<0.0001$). This was also true when comparing only melancholic rMDD patients to the HC group ($t(69)=4.06$, $p(\text{unc})<0.0001$). Despite this, the mean scores for both the rMDD patients (both overall and melancholic) were within the range of mild subthreshold depressive symptoms (Beck *et al*, 1988). Furthermore, the groups did not differ with respect to current Montgomery-Åsberg Depression Rating Scale (MADRS) scores. The melancholic and non-melancholic rMDD patients did not differ with respect to age, sex, number of MDEs, age of onset, months since remission, months since last psychotropic use, number of patients previously treated, number of suicide attempts, or family history of MDD. However, relative to the non-melancholic rMDD group, melancholic patients had more years of education ($t(61)=2.61$, $p(\text{unc})=0.01$) and their last and most severe MDE was longer in duration ($t(61)=2.41$, $p(\text{unc})=0.02$) with MADRS scores indicating greater severity ($t(61)=3.28$, $p(\text{unc})=0.002$). These potential confounders were controlled for in subsequent analyses (see below).

Image Acquisition

Resting-state echo-planar images (EPI) were acquired on a 3T Philips Achieva MRI scanner (Philips Medical Systems, Best, the Netherlands) with an 8-channel coil and were optimized for the detection of ventral frontal signal (240 volumes; 40 axial slices; 3mm slice thickness; ascending sequential acquisition; repetition time: 2000ms; echo time: 22ms; field of view: 240x240x120mm; acquisition matrix: 80x80 voxels; reconstructed voxel size:

3mm³; flip angle: 90°). Participants were instructed to lie still with eyes closed and to remain awake and were debriefed after scanning to ensure adherence to the instructions, at which time no participants reported having fallen asleep. A 3-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient-echo (MPRAGE) structural image was also obtained for each participant (160 axial slices; 0.9mm slice thickness; repetition time: 8.4ms; echo time: 3.9ms; field of view: 240x191x144mm; acquisition matrix: 256x163 voxels; reconstructed voxel size: 0.94x0.94x0.9mm; flip angle: 8°). For further clinical assessment, T2-weighted structural images were also acquired.

Seed Region Selection

The seed-based rsfMRI analyses were conducted using an *a priori* SCC region of interest (ROI; Montreal Neurological Institute [MNI] coordinates: -4, 23, -5; 6mm sphere) which demonstrated functional decoupling during a task evoking feelings of guilt in patients vulnerable to experiencing MDEs (Green *et al*, 2012). The seed region used in the current study (MNI coordinates: -4, 23, -5) is close in proximity to an SCC region identified in a separate study as demonstrating resting-state functional disconnection in patients vulnerable to MDD (MNI coordinates: 2, 23, -6; (Herrington *et al*, 2013)). Taken together, the seed region used in this study was selected because of its association with depression vulnerability. Furthermore, although located more medially, the seed region's coordinates are close to SCC regions that demonstrated greater connectivity in current MDD patients relative to HC participants (Greicius *et al*, 2007; Sheline *et al*, 2010).

Resting-state fMRI Analysis

EPIs and MPRAGE images were preprocessed using the Artifact Detection Tools (ART; <http://web.mit.edu/swg/software.htm>), DPARSF Advanced Edition ((Chao-Gan and

Yu-Feng, 2010); <http://rfmri.org/DPARSF>), and SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>) MATLAB (MathWorks) toolboxes. SPM8 was used for preprocessing to ensure compatibility with DPARSF. The first 10 volumes of the EPIs were discarded prior to slice timing and head motion correction. ART was used to produce regressors of high-motion volumes (i.e., framewise signal intensity > three standard deviations from the global mean, framewise head displacement > 1mm). The MPRAGE images were co-registered to the EPIs and segmented. EPIs underwent linear detrending and nuisance covariates regression (24 motion parameters (Friston *et al*, 1996), white matter signal, cerebrospinal fluid signal, and ART regressors), normalization using nonlinear transformation parameters derived during segmentation, and smoothing using a 6mm full-width at half-maximum Gaussian kernel, prior to band-pass filtering to retain frequencies between 0.01Hz and 0.08Hz. Volume censoring was then performed to remove high-motion volumes identified by ART and resulting segments of uncensored data comprised of fewer than five contiguous volumes. All resulting EPIs contained \geq five minutes of data (\geq 150 volumes).

Functional connectivity maps were computed using the resultant EPIs for each participant by correlating the average time course within the seed region with the time course of each voxel within the brain. The functional connectivity maps were then Fisher Z-transformed and entered into an analysis of variance (ANOVA) in SPM12 because it allows for cluster-level familywise error (FWE) correction of *F*-tests for differences between melancholic rMDD, non-melancholic rMDD, and HC groups. Significance was determined using an uncorrected voxel-level cluster forming threshold of $p < 0.001$ and a cluster-level FWE-corrected threshold of $p < 0.05$ across the whole-brain ($p < 0.05$) or *a priori* ROIs ($p < 0.007$ corresponding to approximate Bonferroni-corrected $p < 0.05$ for the seven ROIs used in our analyses). The following bilateral *a priori* ROIs were defined: ventromedial prefrontal cortex, dorsomedial prefrontal cortex, anterior temporal cortex, amygdala, hippocampus,

septal region, and hypothalamus. The definition of these ROIs was described previously (Zahn *et al*, 2009) except for the hippocampal ROI which combined the left and right hippocampus masks from the Automatic Anatomical Labeling atlas (Tzourio-Mazoyer *et al*, 2002). These ROIs were chosen because of their close structural and/or functional connections with the SCC (Carmichael *et al*, 1996; Johansen-Berg *et al*, 2008; Kondo *et al*, 2003; Vogt *et al*, 1987), their role in the pathophysiology of depression (Elliott *et al*, 2011; Green *et al*, 2012; Mayberg, 2003), as well as in social cognitive and motivational processes (Elliott *et al*, 2011; Moll *et al*, 2005; Zahn *et al*, 2009). Mean Fisher Z-transformed correlation coefficients for each group were extracted from all clusters surviving FWE-correction and entered into a one-way ANOVA in SPSS 20 (SPSS Inc., Chicago, Illinois, USA) to probe pairwise post-hoc comparisons between groups at Bonferroni-corrected $p < .05$. The results of all statistical tests reported herein are two-tailed.

RESULTS

A main effect of group was observed across the melancholic rMDD, non-melancholic rMDD, and HC groups for connectivity between the SCC seed region and the left amygdala and right parahippocampal gyrus (Table 4; Figure 1).

This was also reflected in significant effects for the extracted cluster averages in both regions (left amygdala: $F(2,98)=11.2$, $p(\text{unc}) < 0.0001$; right parahippocampal gyrus: $F(2,98)=13.5$, $p(\text{unc}) < 0.0001$). Bonferroni-corrected post-hoc pairwise comparisons revealed decreased SCC connectivity with the left amygdala in the melancholic rMDD patients ($M=0.11$, $SD=0.16$) compared to both the non-melancholic rMDD ($M=0.25$, $SD=0.13$, $p=0.001$, mean difference=-0.14, 95% CI [-0.22,-0.05]) and HC groups ($M=0.25$, $SD=0.13$, $p < 0.0001$, mean difference=-0.14, 95% CI [-0.22,-0.06]). The same pattern of distinctively

decreased SCC connectivity in melancholic rMDD emerged for the right parahippocampal gyrus (M=0.13, SD=0.14) compared with the non-melancholic rMDD (M=0.23, SD=0.15, $p=0.013$, mean difference=-0.11, 95% CI [-0.20,-0.02]) and HC groups (M=0.31, SD=0.15, $p<0.0001$, mean difference=-0.18, 95% CI [-0.26,-0.09]).

SCC connectivity across the rMDD patients was not associated with the number of previous MDEs (left amygdala: $r_s = -0.005$, $p(\text{unc})=0.97$; right parahippocampal gyrus: $r_s = 0.10$, $p(\text{unc})=0.43$). Across the melancholic and non-melancholic rMDD groups, we found no evidence for effects of potentially confounding variables unrelated to subtype since SCC connectivity was neither associated with years of education (left amygdala: $r_s = -0.005$, $p(\text{unc})=0.97$; right parahippocampal gyrus: $r_s = 0.10$, $p(\text{unc})=0.43$) nor current BDI scores (left amygdala: $r_s = -0.08$, $p(\text{unc})=0.55$; right parahippocampal gyrus: $r_s = -0.06$, $p(\text{unc})=0.67$). Of note, there were no group differences in mean framewise displacement, a measure of relative head displacement between contiguous volumes (Power *et al*, 2012)(Table 2).

In further analyses, we investigated whether categorical differences between rMDD subtypes in SCC connectivity could be dissociated from differences in past MDE severity and duration that are closely associated with the definition of melancholic versus non-melancholic MDD. As expected, patients with longer past episode duration showed decreased SCC connectivity with both regions (left amygdala: $r_s = -0.30$, $p(\text{unc})=0.02$; right parahippocampal gyrus: $r_s = -0.32$, $p(\text{unc})=0.01$) and there was a trend towards patients with more severe past episodes to show decreased SCC connectivity with the left amygdala ($r_s = -0.23$, $p(\text{unc})=0.07$). SCC connectivity was not, however, associated with past episode duration in the melancholic (left amygdala: $r_s = -0.21$, $p(\text{unc})=0.24$; right parahippocampal gyrus: $r_s = -0.29$, $p(\text{unc})=0.10$) or non-melancholic (left amygdala: $r_s = -0.10$, $p(\text{unc})=0.61$; right parahippocampal gyrus: $r_s = -0.18$, $p(\text{unc})=0.34$) rMDD groups alone. There was a trend towards melancholic patients with more severe past MDEs to show increased connectivity

with the right parahippocampal gyrus ($r_s=0.33$, $p(\text{unc})=0.06$) but not with the left amygdala ($r_s=0.02$, $p(\text{unc})=0.92$), and no such relationship was observed in the non-melancholic patients (left amygdala: $r_s=-0.27$, $p(\text{unc})=0.15$; right parahippocampal gyrus: $r_s=-0.28$, $p(\text{unc})=0.13$). Taken together, this shows that a relationship between SCC connectivity and past episode duration may be a general feature of rMDD, but that this is not specific to either of the subtypes reported here. Additional exploratory analyses further showed that categorical differences in SCC connectivity between subtypes remained when adjusting for the effects of the duration (group difference adjusted for duration: left amygdala: $t(60)=2.93$, $p(\text{unc})=0.005$; right parahippocampal gyrus: $t(60)=2.24$, $p(\text{unc})=0.03$) and severity (group difference adjusted for severity, left amygdala: $t(60)=3.16$, $p(\text{unc})=0.002$) of past episodes.

DISCUSSION

As predicted, patients vulnerable to melancholic MDEs demonstrated distinctive patterns of resting-state functional disconnection with the SCC when compared with non-melancholic rMDD patients. This extends previous work showing that SCC metabolism is distinctively altered in current melancholic MDD (Pizzagalli *et al*, 2004). Intriguingly, contrary to our more specific predictions, we found SCC disconnection with the amygdala and parahippocampal gyrus rather than medial frontal cortices to be distinctive of melancholic rMDD. These group differences in functional connectivity were not due to potentially confounding effects of education, residual symptoms, or the duration and severity of past depressive episodes. Of note, the melancholic and non-melancholic rMDD patients did not differ with respect to previous treatment for MDD, further strengthening the interpretation of group differences in SCC connectivity as correlates of subtype-specific vulnerability.

Our finding of SCC functional disconnection from the amygdala in melancholic rMDD is in agreement with reports of abnormal connectivity and activation of these structures in current MDD and MDD vulnerability. Lower functional connectivity between the SCC and amygdala was associated with vulnerability to experiencing depression in a healthy female cohort (Herringa *et al*, 2013). Kruschwitz and colleagues (2014) recently demonstrated aberrant functional connectivity of the amygdala in individuals homozygous for the short allele of the 5-HTTLPR/rs25531 polymorphism, and the 5-HTTLPR polymorphism is known to confer depression risk. Interestingly, a support vector classification model incorporating resting-state connectivity of regions including the SCC and amygdala has shown promise for distinguishing patients with current MDD from a HC group (Craddock *et al*, 2009). Relatedly, SCC and amygdala reactivity to emotional stimuli was predictive of response to cognitive behavioural therapy in a cohort of MDD patients (Siegle *et al*, 2006). Patients with current MDD also exhibited microstructural white matter abnormalities between the SCC and amygdala (Cullen *et al*, 2010). Resting-state blood flow to the amygdala was associated with a negative emotional bias in rMDD patients following acute tryptophan depletion (Roiser *et al*, 2009). Amygdala activation in response to emotional faces has been widely reported as abnormal in current MDD (reviewed in (Elliott *et al*, 2011)) and its activation was associated with shame experiences in remitted MDD (Pulcu *et al*, 2014).

Our finding of SCC disconnection from the parahippocampal gyrus is in keeping with the known importance of medial temporal lobe structures in MDD. Situated in close proximity to both the amygdala and hippocampus, the parahippocampal gyrus is anatomically connected both to medial temporal structures (Amaral and Price, 1984) and the SCC (via the rostral cingulate (Vogt *et al*, 1987)). In agreement with our findings, lower resting-state SCC-hippocampal connectivity was described in a study of adolescents vulnerable to MDD (Herringa *et al*, 2013). Aberrant functional connectivity of the parahippocampal gyrus was

also reported in rMDD patients during a sad mood induction paradigm (Zamoscik *et al*, 2014). Studies employing local measures of connectivity such as regional homogeneity consistently describe reductions in these measures in the parahippocampal gyrus in current MDD (reviewed in (Dutta *et al*, 2014)). In healthy volunteers, the parahippocampal gyrus has been shown to play a role in representing visual imagery (Downing *et al*, 2006) and in the retrieval of episodic, including autobiographical, memories (Gardini *et al*, 2006).

The network of decreased subgenual-amygdala-parahippocampal connectivity emerging from this study corresponds well to the limbic compartment of Mayberg's (2003) limbic-cortical model of MDD. Mayberg suggests dysfunction within a limbic-cortical network is crucial in understanding the heterogeneity of MDD. In demonstrating disconnection within the limbic compartment associated with vulnerability to a specific MDD subtype, our findings support and extend this view. Notably, the limbic compartment in Mayberg's (2003) model is associated with somatic and vegetative symptoms, which are commonly present in melancholic MDD.

Our finding that melancholic rMDD patients demonstrate decreased SCC connectivity runs counter to rsfMRI studies in current MDD reporting normalization with treatment of initially elevated SCC connectivity (Dutta *et al*, 2014; Greicius *et al*, 2007; Liston *et al*, 2014; Sheline *et al*, 2010). This discrepancy may reflect methodological differences across studies. For example, our seed region is located medially to the subgenual areas captured in rsfMRI studies in current MDD (Greicius *et al*, 2007; Sheline *et al*, 2010). This is an unlikely source of discrepancy, however, given that elevated resting-state activation of subgenual cingulate/septal areas is reported across studies in current MDD using a variety of imaging modalities and analysis techniques (reviewed in (Drevets *et al*, 2008)). A more probable explanation for the decreased SCC connectivity in the melancholic rMDD patients is that studies reporting normalization of SCC connectivity with treatment have been conducted in

recently recovered patients instead of patients in full remission for several months as studied here (Liston *et al*, 2014). Indeed, the decreased connectivity we report in the melancholic rMDD patients is in keeping with a previous study in a cohort also vulnerable to depression which reported resting-state disconnection of the SCC (Herringa *et al*, 2013). Our results are also in keeping with a recent study which demonstrated decreased effective connectivity at rest in current melancholic MDD patients (Hyett *et al*, 2015). Furthermore, these results are in agreement with previous work showing guilt-selective decreases in functional connectivity in a rMDD group which included a large proportion of melancholic patients (Green *et al*, 2012). Given that MDD represents a lifetime diagnosis, this suggests abnormal SCC connectivity is a trait marker for melancholic MDD where the direction of connectivity is state-dependent.

The following limitations of our study need to be discussed: It could be argued that use of a seed-based approach to analyze the rsfMRI data represents a limitation of the current study given the *a priori* assumptions required for seed selection (e.g., see (Dutta *et al*, 2014)). However, SCC dysfunction in MDD is well-established and using a SCC seed provides a direct link to the neuroimaging literature in MDD, including the study in rMDD from which the seed region was selected (Green *et al*, 2012). Another potential limitation of the current study is that we cannot firmly conclude whether decreased connectivity is associated with primary vulnerability which precedes the initial onset of MDD or secondary vulnerability where “scarring” increases vulnerability to subsequent MDEs (Burcusa *et al*, 2007). The lack of correlation between connectivity and number of previous MDEs, however, renders an association with primary vulnerability more likely. Another limitation to the current study is that we did not explicitly control for physiological artefacts associated with breathing and heart-rate. Although such artefacts predominantly affect high frequencies not typically investigated with rsfMRI, changes to breathing or heart-rate also appear as low-frequency

fluctuations that may correlate with changes to BOLD signal thereby spuriously increasing or creating patterns of functional connectivity (Murphy *et al*, 2013). We did, however, regress out white matter and cerebrospinal fluid signals which are thought to reflect such physiological artefacts (Dagli *et al*, 1999; Windischberger *et al*, 2002). Other strict controls for motion were also employed, such as regressing out an expanded set of 24 motion parameters (Friston *et al*, 1996; Power *et al*, 2014) and removing high-motion volumes (e.g., (Power *et al*, 2014)) identified with the ART toolbox.

Taken together, the present study successfully used a SCC seed-based rsfMRI approach to identify patterns of decreased connectivity distinctive of melancholic rMDD. Melancholic rMDD patients demonstrated decreased connectivity of the SCC with the amygdala and parahippocampal gyrus when compared with the non-melancholic rMDD and HC groups. These results provide the first resting-state neural signature distinctive for melancholic rMDD and may reflect a subtype-specific primary vulnerability factor for MDD. Longitudinal investigations of patients with a positive family history prior to their first MDE could be used to further validate these results as a biomarker of primary vulnerability to melancholic MDD.

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Supplementary information is available at the *Neuropsychopharmacology* website.

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FIGURE LEGENDS

Figure 1. a) The network of regions demonstrating resting-state functional disconnection with the subgenual cingulate seed region in the remitted melancholic MDD patients when compared to the remitted non-melancholic MDD and HC groups. Whole-brain images were cropped and displayed at an uncorrected voxel-level threshold of $p < 0.001$. b) Bar plots showing group differences in average Z-transformed correlation coefficients and standard errors for the right parahippocampal gyrus and left amygdala clusters. AMYG, amygdala; HC, healthy control; L, left; MDD, major depressive disorder; PHG, parahippocampal gyrus; R, right; SCC, subgenual cingulate cortex.

Tables

Subgenual cingulate-amygdala functional disconnection and vulnerability to melancholic depression

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Table 1. Reasons for exclusion of volunteers from the current study

Reasons for Telephone Pre-Screening Exclusions	<i>N</i>
MRI contraindications	77
Psychiatric disorders other than MDD	54
Current antidepressants or other centrally active medications	52
Withdrawal after telephone pre-screening	33
Not meeting full screening criteria for MDD	30
Family history of MDD/bipolar/schizophrenia (HC group)	26
Substance or alcohol abuse	23
Current antihypertensive or statin medications	20
Left-handed	20
Non-native English speaker	19
Thyroid function problems	19
Fulfilling criteria for current MDD	13
History of cancer	7
Not remitted for long enough (≥ 6 months)	7
Epilepsy	5
No reason recorded	5
Other general medical conditions	5
Diabetes	4
Out of age range (18 – 65 years)	4
Excluded because of age-matching (HC group)	3
Multiple sclerosis	3
History of stroke	1
Vitamin D deficiency	1
<i>Total excluded after the telephone pre-screening</i>	<i>431 / 707</i>
Reasons for Clinical Interview Exclusions (remitted MDD patients)	<i>N</i>
Unable to schedule for additional visits	10
Fulfilling criteria for a bipolar disorder	6
Fulfilling criteria for current social anxiety disorder	6
Not meeting full criteria for MDD	5
Fulfilling criteria for past substance abuse	4

Not remitted for long enough (≥ 6 months)	3
Residual symptoms of post-traumatic stress disorder	3
Probable personality disorders	2
Fulfilling criteria for current generalized anxiety disorder	1
MRI contraindications	1
Withdrawal after the clinical interview	1
<i>Total number of remitted MDD patients excluded after the clinical interview</i>	<i>42 / 138</i>
Reasons for Clinical Interview Exclusions (HC group)	<i>N</i>
Unable to schedule for additional visits	6
Probable or definite positive first degree family history of MDD	4
Fulfilling criteria for a past MDE lasting less than two months	1
Fulfilling criteria for current adjustment disorder	1
Fulfilling criteria for current MDD	1
Fulfilling criteria for current social anxiety disorder	1
Non-native English speaker	1
Past depressive episode not fulfilling criteria for a past MDE	1
<i>Total number of HC participants excluded after the clinical interview</i>	<i>16 / 64</i>

Of the 707 volunteers who completed the telephone pre-screening, 276 were eligible (184 remitted MDD patients, 92 HC participants). Of these, 202 participants agreed to complete the clinical interview after having reviewed the study's participant information sheet (138 remitted MDD patients, 64 HC participants). Following the clinical interview, 144 participants were eligible to complete the remaining study visits (96 remitted MDD patients, 48 HC participants). Of these, 102 participants underwent resting-state fMRI scanning (63 remitted MDD patients, 39 HC participants). fMRI, functional magnetic resonance imaging; HC, healthy control; MDD, major depressive disorder; MDE, major depressive episode.

Table 2. Demographic variables in the remitted MDD and HC groups

Demographic Variables	HC (N=38)		rMDD (N=63)		Melancholic rMDD (N=33)		Non-Melancholic rMDD (N=30)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	36.2	13.8	36.4	12.3	37.7	11.1	35	13.4
Years of Education ¹	16.8	2.3	16.8	2.4	17.5	2.2	16	2.4
BDI Score ²	0.9	1.7	3.6	4.1	3.9	4.1	3.2	4.2
MADRS Score	0.7	1.3	0.8	1.4	0.7	1.3	0.9	1.6
Sex (Male/Female)	13/25		22/41		9/24		13/17	
Framework Displacement (mm)	0.24	0.15	0.27	0.25	0.26	0.15	0.29	0.33

With the exception of BDI scores, the remitted MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient<0.008, $p(\text{unc})>0.94$; $t<0.79$, $p(\text{unc})>0.43$). Also with the exception of BDI scores, the remitted melancholic MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient<0.08, $p(\text{unc})>0.52$; $t<1.30$, $p(\text{unc})>0.20$). With the exception of Years of Education, the remitted melancholic and non-melancholic MDD patients did not significantly differ on the demographic variables (Contingency Coefficient<0.17, $p(\text{unc})>0.18$; $t<0.89$, $p(\text{unc})>0.38$). BDI, Beck Depression Inventory; HC, healthy control; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; SD, standard deviations.

¹Significantly different between the remitted melancholic and non-melancholic MDD groups ($t(61)=2.61$, $p(\text{unc})=0.01$).

²Significantly different between the remitted MDD and HC groups ($t(99)=3.71$, $p(\text{unc})<0.0001$), and between the remitted melancholic MDD and HC groups ($t(69)=4.06$, $p(\text{unc})<0.0001$).

Table 3. Clinical characteristics of the remitted melancholic and non-melancholic MDD patients

Clinical Characteristics	Melancholic MDD (N=33)		Non-Melancholic MDD (N=30)	
	Mean	SD	Mean	SD
Number of previous MDEs				
1	8/33		10/30	
2	9/33		7/30	
3	8/33		6/30	
4	3/33		3/30	
5	4/33		2/30	
6 or more	1/33		2/30	
Average number of previous MDEs	2.8 (range: 1–9)	1.7	3.8 (range: 1–44)	7.7
Age of onset	23.9 (range: 11–49)	9.9	21.0 (range: 8–44)	9.4
Last and most severe MDE details	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
Average length of MDE (months) ¹	20.6 (range: 2–96)	23.5	9.6 (range: 1–36)	9.0
Average time in remission (months)	30.9 (range: 6–140)	28.6	34.6 (range: 6–282)	51.0
Average MADRS score for MDE ²	37.3 (range: 26–48)	5.1	32.8 (range: 20–44)	5.9
No psychotropic medication since (months)	53.1 (range: 2–372)	73.6	48.0 (range: 4–282)	64.0
Previous treatment	<i>N</i>		<i>N</i>	
SSRI antidepressant	29/33		22/30	
SNRI antidepressant	1/33		1/30	
Tricyclic antidepressant	4/33		1/30	
Mirtazapine	0/33		1/30	
Unknown class of antidepressant	5/33		3/30	
Benzodiazepines only	0/33		1/30	
No antidepressant medication	2/33		2/30	
CBT	10/33		4/30	
Self-guided CBT via internet, books	3/33		1/30	
Hypnotherapy	0/33		1/30	
Counselling	17/33		12/30	
Suicide attempts	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
	0.09 (range: 0-1)	0.29	0.40 (range: 0–5)	1.10
Lifetime axis-I comorbidity³	<i>N</i>		<i>N</i>	
Panic disorder with agoraphobia	1/33		0/30	
Bulimia nervosa	1/33		0/30	
Post-traumatic stress disorder	1/33		0/30	

No life-time co-morbidity	30/33	30/30
Family history	<i>N</i>	<i>N</i>
First degree relative with MDD	17/33	20/30
No family member with history of MDD	13/33	9/30
First degree relative with schizophrenia or bipolar disorder	3/33	1/30

All MDD participants stopped medication before the required washout phase. Means and standard deviations are reported and/or the number of cases. Remitted melancholic and non-melancholic MDD participants did not significantly differ on number previous episodes, age of onset, average time in remission, average time since last taking psychotropic medications, number of patients previously treated, number of suicide attempts, lifetime axis-I comorbidity, or family history (Contingency Coefficient <0.21 , $p(\text{unc})>0.09$; $t<1.55$, $p(\text{unc})>0.13$). Furthermore, there were no differences between the remitted melancholic and non-melancholic MDD participants regarding previous treatment with SSRIs, SNRIs, tricyclics, mirtazapine, or CBT (Contingency Coefficient <0.21 , $p(\text{unc})>0.10$). CBT, cognitive behavioural therapy; MDD, major depressive disorder; MDE, major depressive episode; SD, standard deviations; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor.

¹Significantly different between the remitted melancholic and non-melancholic MDD groups ($t(61)=2.41$, $p(\text{unc})=0.02$).

²Significantly different between the remitted melancholic and non-melancholic MDD groups ($t(61)=3.28$, $p(\text{unc})=0.002$).

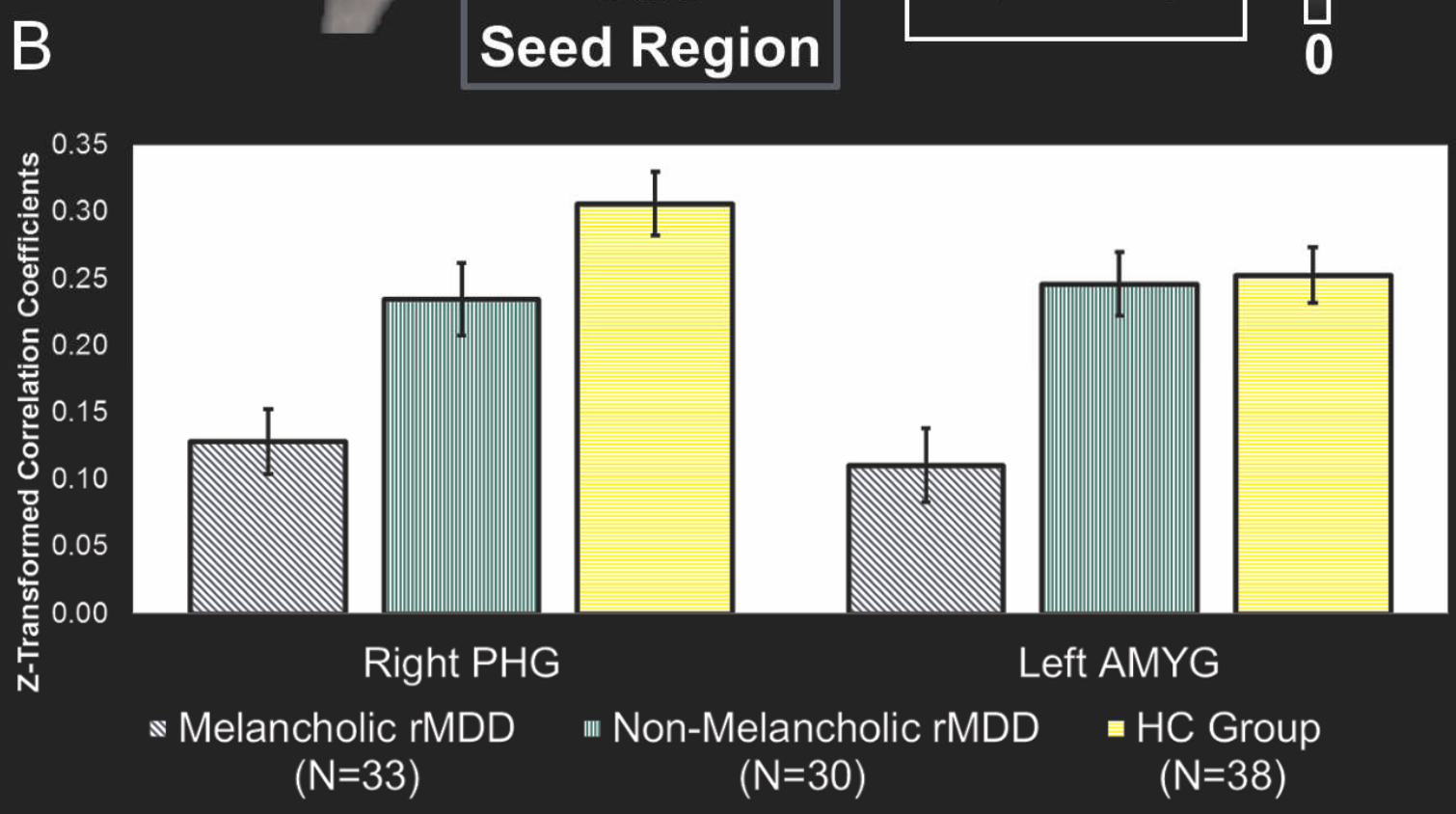
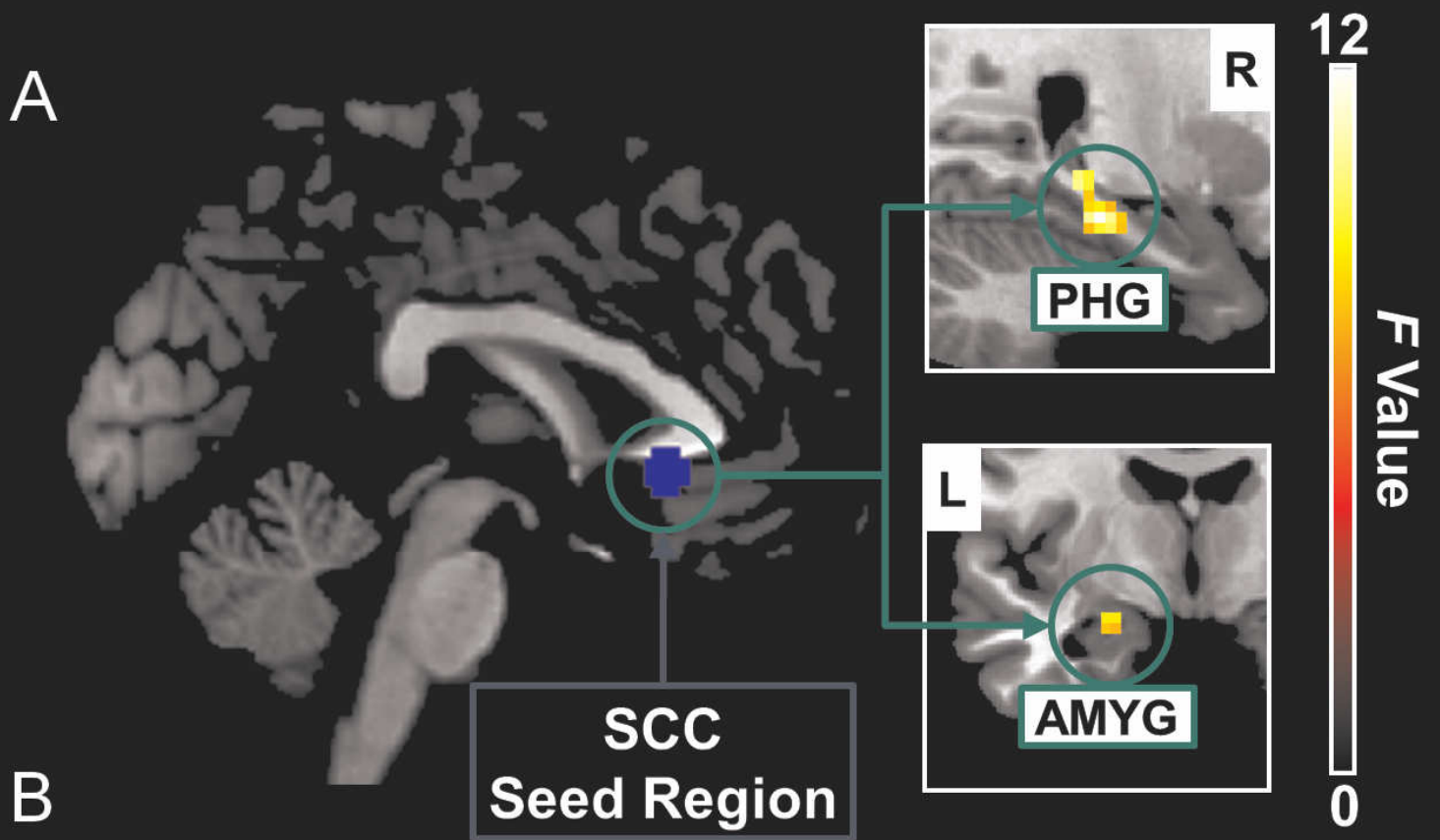
³All co-morbid disorders were fully remitted at the time of study and none were likely to be the primary cause of the depressive episodes.

Table 4. Resting-state functional disconnection in the remitted melancholic MDD patients vs the non-melancholic patients and HC group

Hemisphere	Regions	Peak MNI Coordinates			Peak z Score	Cluster Size	FWE-Corrected p Value
		X	Y	Z			
FWE-corrected at the cluster-level over the whole brain ($p < 0.05$)							
R	Parahippocampal gyrus	24	-27	-15	4.22	52	0.045
FWE-corrected at the cluster-level over an <i>a priori</i> amygdala ROI ($p < 0.007^1$)							
L	Amygdala	-24	-6	-15	3.54	10	0.006

FWE, familywise error; HC, healthy control; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; R, right; ROI, region of interest.

¹Corresponding to approximate Bonferroni corrected $p < 0.05$ /number of ROIs to correct for the number of ROIs used in the study.



Supplementary Information

Subgenual cingulate-amygdala functional disconnection and vulnerability to melancholic depression

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SUPPLEMENTARY RESULTS

The melancholic and non-melancholic remitted major depressive disorder (MDD) patients did not differ on a number of demographic and clinical variables including age, sex, number of major depressive episodes (MDE), age of onset, months since remission, months since last psychotropic use, number of suicide attempts, and family history of MDD. It could be argued that the lack of significant group differences we observed for these variables might be attributable to large variability, which could in turn be associated with the group differences in subgenual cingulate cortex (SCC) connectivity to the left amygdala and right parahippocampal gyrus we reported. However, across all remitted MDD (rMDD) patients, SCC connectivity was not associated with age (left amygdala: $r_s=-0.15$, $p(\text{unc})=0.25$; right parahippocampal gyrus: $r_s=0.06$, $p(\text{unc})=0.66$), number of MDEs (left amygdala: $r_s=-0.09$, $p(\text{unc})=0.50$; right parahippocampal gyrus: $r_s=-0.07$, $p(\text{unc})=0.57$), age of onset (left amygdala: $r_s=-0.14$, $p(\text{unc})=0.29$; right parahippocampal gyrus: $r_s=0.03$, $p(\text{unc})=0.84$), months since remission (left amygdala: $r_s=-0.16$, $p(\text{unc})=0.20$; right parahippocampal gyrus: $r_s=-0.03$, $p(\text{unc})=0.81$), months since last psychotropic use (left amygdala: $r_s=-0.21$, $p(\text{unc})=0.12$; right parahippocampal gyrus: $r_s=-0.07$, $p(\text{unc})=0.60$), or number of suicide

attempts (left amygdala: $r_s=0.08$, $p(\text{unc})=0.52$; right parahippocampal gyrus: $r_s=-0.09$, $p(\text{unc})=0.46$). Furthermore, rMDD patients with a family history of MDD did not differ from patients without a family history on SCC connectivity to the left amygdala (family history of MDD: $M=0.16$, $SD=0.14$; no family history: $M=0.17$, $SD=0.18$; $t(57)=0.20$, $p(\text{unc})=0.84$) or to the right parahippocampal gyrus (family history of MDD: $M=0.17$, $SD=0.14$; no family history: $M=0.19$, $SD=0.18$; $t(57)=0.46$, $p(\text{unc})=0.65$). Male rMDD patients did, however, demonstrate increased connectivity relative to female patients between the SCC and left amygdala (male: $M=0.23$, $SD=0.16$; female: $M=0.14$, $SD=0.15$; $t(61)=2.23$, $p(\text{unc})=0.03$) but not with the right parahippocampal gyrus (male: $M=0.22$, $SD=0.18$; female: $M=0.16$, $SD=0.13$; $t(61)=1.51$, $p(\text{unc})=0.14$). Within the male subgroup, melancholic rMDD patients demonstrated decreased SCC-amygdala connectivity relative to non-melancholic patients (melancholic rMDD patients: $M=0.14$, $SD=0.18$; non-melancholic patients: $M=0.30$, $SD=0.12$; $t(20)=2.53$, $p(\text{unc})=0.02$). The same pattern was observed within the female subgroup (melancholic rMDD patients: $M=0.10$, $SD=0.15$; non-melancholic patients: $M=0.21$, $SD=0.13$; $t(39)=2.35$, $p(\text{unc})=0.02$). Taken together, the patterns of SCC-amygdala connectivity we observed within the male and female subgroups were consistent with our finding of decreased SCC-amygdala connectivity in melancholia. This confirms that differences between melancholic and non-melancholic rMDD groups with respect to SCC-amygdala connectivity did not interact with sex differences.

Table S1. Inter-rater reliability for SCID-I subtype diagnoses and for MADRS scores

Inter-Rater Reliability	SCID-I subtype	MADRS (current)	MADRS (previous MDE)
Raters	Kappa Value	ICC Value	ICC Value
RZ & KL	0.60	0.63	0.45
RZ & JG	–	0.91	0.80
KL & JG	1.00	0.86	0.80
Mean	0.80	0.80	0.68

Reliability for the SCID-I mood disorders module subtype diagnosis is given as a kappa value. Reliability for the MADRS is given as an intra-class correlation (ICC) value (two-way mixed with absolute agreement). RZ, KL, and JG completed the recommended training for the SCID-I for DSM-IV-TR. The SCID-I was modified to allow lifetime diagnoses of MDD subtypes, including melancholic and atypical specifiers. The MADRS was used to assess depression severity at the time of the clinical interview, and was modified to allow for retrospective assessment of the last and most severe MDE. The Kappa values for the SCID-I subtype diagnoses reflect moderate to perfect agreement (Landis and Koch, 1977), and ICC values for the MADRS (both current and previous MDE) reflect moderate to excellent agreement (Fleiss, 1986). ICC, intra-class correlation; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; MDE, major depressive episode; SCID-I, Structured Clinical Interview-I.

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