Online-Only Material

Guilt-selective functional disconnection of anterior temporal and subgenual cortices in major depressive disorder

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Online-Only methods

Participant selection and description

Inclusion and exclusion criteria

Inclusion criteria for both groups were: right handedness, MRI eligibility criteria, English as first language and age between 18-65 years. Additional inclusion criteria for the remitted major depressive disorder (MDD) group were at least one past major depressive episode according to Diagnostic Statistical Manual-IV-TR [1], that was a moderate to severe depressive episode according to the International Classification of Diseases (ICD-10, [2]) lasting at least 2 months, requiring treatment, and remission of symptoms for at least 12 months.

Exclusion criteria for both groups were: residual symptoms of or manifest axis-I disorders [1], significant psychosocial impairment as an indicator of a clinically relevant personality disorder or incomplete remission, a Montgomery Asberg Depression Rating Scale ([3], MADRS) score >10 (=cut-off for depression), current self-harming behaviour, an abnormal MRI scan, a history of alcohol or substance abuse, schizophrenia, schizo-affective disorder, bipolar disorder, developmental disorders, learning disabilities, neurological illnesses (MRI scan & neurological examination) or physical illnesses (clinical history) that significantly impair psychosocial functioning, brain function or blood flow. Participants were also excluded if they selected more than one feeling on more than 5% of the trials of the post-scanning rating indicating non-compliance with the instructions.

Additional exclusion criteria for the remitted MDD group were: centrally active medication other than antidepressants or hormonal contraceptives, or depressive episodes secondary to another psychiatric disorder. Additional exclusion criteria for the healthy control group were: centrally active medication other than hormonal contraceptives, a history of medication with antidepressants, antipsychotics, or tranquilizers, or a first degree relative with a diagnosed major depression, bipolar disorder or schizophrenia, or a history of any axis-I disorder with a corresponding category in ICD-10.

Participant screening

Participants' suitability for the study was first assessed using a phone pre-screening interview (see Appendix). The screening questions for MDD, alcohol and substance abuse were taken from the International Neuropsychiatric Interview [4]. The screening questions for other major psychiatric disorders including bipolar disorder, schizophrenia, obsessive compulsive disorder, post-traumatic stress disorder and borderline personality disorder, were based on clinical experience indicating that these questions provide high sensitivity and selectivity for these disorders. The

questions for inclusion into MDD groups were taken from the melancholic subtype questions of the SCID-I [1] in order to select for severe forms of MDD and against milder and differential diagnostically less valid forms of MDD (see eTable 8 for details of the 171 volunteers pre-screened for the study and selection of the final sample).

General clinical characteristics of final groups

In the remitted MDD group, N=11/25 were using hormonal contraceptives, N=1/25 was taking hormonal stimulation medication, and N=13/25 had no hormonal contraception (for further clinical characteristics of the MDD group see eTable 7 and eTable 9).

In the control group, none took centrally active medication other than hormonal contraceptives (N=13/22). 2/22 control participants had a first degree relative who had taken antidepressant medication but received no diagnosis of MDD, 1/22 had a first degree relative with obsessive thoughts but who was untreated and had no diagnosis of obsessive compulsive disorder. In 19/22 of the control participants first degree relatives with psychiatric diagnoses could be ruled out with high certainty.

Behavioural data analysis

Analysis of between-group differences were performed using 2-sided two-sample t-tests at p=.05 in SPSS15 (www.spss.com). As in our previous studies [5, 6], trials were only included in both the imaging analysis and behavioural analysis if participants selected guilt in the self-agency condition and indignation in the other-agency condition.

Imaging methods

Imaging procedures

Stimuli were presented using the presentation software, E-prime version 1.1 (http://www.pstnet.com/) and were projected from the control room into the scanner room and back-projected from a projection screen to a mirror system above the participants' eyes. Before the fMRI paradigm, participants completed a practice session outside of the scanner so that they were accustomed to the finger-to-response assignment. The practice session used 12 stimuli (6 negative, 6 negated positive) that were not presented during scanning. Two buttons were assigned to two different fingers of the right hand for these responses (finger-to-response assignment randomized across participants). If they responded before the end of the 5 seconds, the stimulus was replaced with a fixation cross for the remaining duration and followed by a

jittered inter-trial interval with a mean duration of 4 seconds (jittered in 9 steps of 500 ms around the mean interval with equal distribution of intertrial intervals across different stimulus types). Stimuli of different conditions were presented in a pseudo-random order across three runs. The order of administration of the runs was randomized across participants.

After scanning, participants rated each fully randomly ordered statement on unpleasantness (***"How strongly would you experience unpleasant feelings?",*** 7-step Likert visual analogue scale with numbering: ***1=not unpleasant, 7=extremely unpleasant***) and were required to "***choose the feeling that (***they) ***would feel most strongly"*** from a choice of: ***guilt, contempt/disgust towards self, shame, indignation/anger towards self, indignation/anger towards other, contempt/disgust towards other, none, other***. Participants were also required to provide a rating of how many possible consequences they estimated from the described social behaviour (***"Please estimate how many different outcomes of the social behaviour there are",*** 7-step Likert visual analogue scale without numbering: 1=***very few – 7=very many***) and asked to rate in how much detail they thought the sentence described social behaviour ***("In how much detail does this statement describe a characteristic set of social behaviours?",*** 7-step visual analogue scale without numbering: 1=***little detail – 7=great detail).*** After that they were presented with the 90 social concepts contained in the stimulus set and were asked to rate "***How well does this word describe you?"*** and "***How well does this word describe your best friend?***" (7-step Likert visual analogue scale without numbering: 1=***not well at all – 7=extremely well***).

Participants completed a final task at home or in a testing lab in which they rated how intensely they visualized the described behaviour ("***How intensely did you visualize the described behaviour?",*** 7-step Likert visual analogue scale without numbering: 1=***not at all – 7=extremely intensely)*** and how much they were reminded of specific autobiographical episodes (***"How intensely were you reminded of a specific episode or scene experienced during your life?",*** modified from [7], 7-step Likert visual analogue scale without numbering: 1=***not at all - 7=extremely).***

Region of Interest (ROI) definition

In order to create tier 1 ROIs we used the Automatic Anatomical Labelling atlas (AAL [8], implemented in the Wake Forrest University (WFU) Pickatlas tool ([9], for details see [6], http://cercor.oxfordjournals.org/cgi/content/full/bhn080/DC1). A frontopolar cortex (BA 10) region was created using the WFU pickatlas tool implementing the Talairach Daemon atlas [10]. A bilateral ROI of the medial temporal lobes was created using the hippocampus and parahippocampal gyrus

masks from the Automated Anatomical Labelling atlas implemented in the WFU pickatlas tool. For tier 2 ROIs, we used the WFU Pickatlas tool [9] and a lateral orbitofrontal cortex ROI with a centre coordinate of x=41, y=33, z=−2 was created as in [5] by averaging the peak coordinates of three independent studies that linked lateral OFC activation with moral indignation/anger [11-13] and drawing a sphere of 6 mm (radius) around this point. An SCSR ROI with a radius of 6 mm was drawn around the coordinate x=-4, y=23, z=-5 (as used in [5]) by averaging the peak coordinates of four separate studies that have implicated this area in the experience of guilt and other pro-social sentiments [6, 12, 14, 15].

Standard BOLD effect analysis

For the BOLD analysis, first level models were created for each person using the trials on which participants rated having felt guilt in the self-agency or indignation/anger towards their best friend in the other-agency condition. Null events (visual fixation trials) were included for the three runs. Random effects analyses for BOLD and PPI analyses both used two-sample t-tests for between-group analyses using the defaults in SPM8 with the same contrasts of ***guilt vs. indignation***, inclusively masked with ***guilt vs. fixation*** and using the same thresholds of a minimum cluster size of 4 voxels with a voxel-wise p=.005 for between-group differences and p=.01 for within-group analyses. We modeled the temporal and spatial derivatives of the hemodynamic response function. All analyses were inclusively masked with a grey matter mask based on the normalized T1-weighted images from our participants. All reported statistics only include voxels surviving this inclusive masking and surviving additional cluster- or voxel-based FWE-correction at p=.05 over either the whole brain or our a priori ROIs.

Grey Matter Mask Generation

The grey matter mask was created by first segmenting all participants' (N=47) T1 images using the New Segment function in SPM8 to produce roughly aligned grey and white matter images. Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) in SPM8 was then used to create a template by aligning all grey matter images at the same time as aligning all white matter images and to spatially smooth (FWHM 6x6x6) and normalize all grey matter images to MNI space. The resulting normalized and smoothed images were then used to create a mean template using the ImCalc function in SPM, and the resulting mask was thresholded at >.1 for the final mask as in [5].

Regional fMRI signal coverage

Of the 22 participants with good coverage of our primary ROIs (see eFigure 3 for the implicit mask for this analysis), there was one participant with signal drop-out in the frontopolar

cortex running through the left dorsolateral frontal cortex. In order to explore effects in these regions, a second analysis was carried out excluding this person (see eFigure 3). Our fMRI sequence provided good coverage of the subgenual cingulate and superior anterior temporal areas and had been optimized according to our previous studies [6, 16] and pilot testing (see eFigure 3). For the second analysis excluding the one participant with signal dropout, a second grey matter mask was created.

Online-Only results

For all imaging analyses, only regions are reported that survived inclusive masking with ***guilt vs. fixation (for guilt vs. indignation)*** and ***indignation vs. fixation (for indignation vs. guilt).***

Between-group BOLD results

Between-group comparisons of ***guilt vs. indignation*** are reported in eTable 2 and summarized in the main manuscript. For ***indignation vs. guilt,*** there were no regions which showed greater activation for controls compared to the MDD group. The MDD group, however, showed increased activation of a posterior insula/superior temporal region and a parieto-occipital region for ***indignation vs. guilt.*** These were the same regions in which the control group showed higher BOLD effects for ***guilt vs. indignation*** compared with MDD. Masking with comparisons against the visual fixation condition for both contrasts ruled out that the observed activations were due to negative BOLD effects in the active conditions.

Within-group standard BOLD results

For the control group the comparison of ***guilt vs. indignation*** resulted in BOLD effects in the following regions: right anterior middle temporal gyrus, bilateral dorsal anterior cingulate cortex, right dorsal paracingulate cortex, right cuneus, left precentral gyrus and left posterior superior temporal gyrus (see eTable 3). There were no significant effects for ***guilt vs. indignation*** in the MDD group or for ***indignation vs. guilt*** in either the control group or the MDD group.

Between-subject differences in standard BOLD results across groups

Individuals with high percentages of guilt-experience during the self-agency condition had higher BOLD responses for ***guilt vs. indignation*** in the subgenual cingulate/septal region (SCSR, left hemispheric peak), the right supragenual anterior cingulate and the left caudate. This was an across-group effect with group modelled as a covariate of no interest in addition to modelling a covariate of percentages of indignation during the other-agency condition to remove variance due to proneness to indignation (see eTable 6).

Between-group PPI results for guilt vs. indignation using rated unpleasantness as a covariate

To corroborate that between-group differences in PPI effects for guilt vs. indignation were not influenced by between-subject differences in rated unpleasantness, we computed an average unpleasantness rating over guilt and indignation trials for each participant. We used this variable as a covariate of no interest in a linear regression model testing for between-group differences on peak voxel PPI effects for guilt vs. indignation in all regions reported in Table 1. Group differences in all regions survived this covariance analysis (SCSR: t[46]=-4.4, p=.00006, partial beta=-.58; medial frontopolar cortex: t[45]=-4.0, p=.0002, partial beta=-.52; hippocampus: t[46]=-5.0, p=.000008, partial beta=-.61; hypothalamus: t[46]=3.4, p=.001, partial beta=-.46) with no significant regression effects of the covariate in any model. In addition we used the mean unpleasantness of guilt and indignation trials for each participant as a covariate of no interest in our SPM model of between-group differences in guilt vs. indignation for PPI effects. This analysis revealed identical clusters of PPI effects as in our original analysis (see eFigure 4).

Between-group PPI results for guilt vs. indignation in remitted MDD subgroup with no medication

To confirm that the main results of this study, the self-blame-selective decoupling effect in the MDD group were reproduced in the subgroup with no current medication, we extracted the PPI regression coefficients (betas) for ***guilt vs. indignation*** from the peak voxels of significant clusters resulting from the group comparisons and carried out a secondary data analysis comparing the betas between the control (N=22) and the remitted MDD subgroup with no medication (N=16). As in our main analysis, compared with the control group, the subgroup with no medication showed lower coupling for ***guilt vs. indignation*** in all the previously shown brain regions (two-sample t-test: SCSR: t=4.2, p=.0002; frontopolar: t=4.1,p=.0003, hypothalamus: t=3.9, p=.0004; hippocampus: t=4.4, p=.0001).

Between-group PPI results for indignation vs. guilt

The comparison of guilt vs. indignation for controls > remitted MDD was reported in the main manuscript results section. For indignation vs. guilt, there were no regions that showed stronger coupling effects with the ATL in the control compared to the MDD group. For MDD vs. controls, however, there was increased coupling for indignation vs. guilt between the ATL and medial frontopolar cortex (BA10), SCSR, lateral hypothalamus, and right hippocampus (see eTable 4). eFigure 2 shows that the between-group differences in SCSR-ATL coupling during indignation trials were partly due to a decrease in coupling compared with the visual fixation baseline in the control group that failed to take place in the MDD group. This resulted in an abnormal lack of SCSR-ATL decoupling in the MDD group during indignation trials together with the abnormal lack

of SCSR-ATL coupling during the guilt trials reported in the main manuscript. eFigure 5 supports this conclusion by showing that in regions, that displayed between-group ATL-coupling differences, control participants exhibited ATL-decoupling in the ***indignation*** condition relative to both ***fixation*** and ***guilt***.

Within-group PPI results

eTable 5 shows the within-group PPI analyses: In the control group, there was higher ATL coupling for ***guilt vs. indignation*** in the following regions: SCSR (left hemisphere peak), dorsomedial paracingulate cortex (right hemisphere peak), right hippocampus, right lateral hypothalamus, left amygdala, and bilateral occipital pole. There were no significant effects for the MDD group for ***guilt vs. indignation.*** For ***indignation vs. guilt*** there were no regions showing significant coupling with the ATL in the control or the MDD group. In summary, there was significant ATL coupling for guilt relative to the other conditions in the control group in all the areas that showed between-group differences compared with MDD (i.e. reduced coupling in the MDD group in the guilt condition). The lack of significant guilt-selective ATL-coupling in the MDD group supports the abnormalities in this network detected on between-group comparisons.

Supporting subgroup analyses

With men having about half the lifetime prevalence of MDD compared with women[17], we would have expected 33% males (N=8) instead of 25% (N=5) as recruited into our MDD group. It therefore appears that men are underrepresented in our study. However, male participants with MDD showed significantly lower SCSR-ATL coupling as compared with male control participants for guilt vs. indignation which rules out that the group difference for MDD vs. controls was solely driven by female participants (SCSR peak voxel from Tab 1: t[7]=3.4, p=.01). There was no effect of number of episodes (F[2,22]=.35, P=.71) or subtype (melancholic / non-melancholic: t[23]=-.52, p=.61) on SCSR coupling (peak voxel from Tab 1) with the ATL for guilt vs. indignation within the MDD group.

Online-Only Discussion

Overall there were little differences in average BOLD responses between groups which is in keeping with a primary abnormality in functional connectivity rather than average BOLD response in remitted MDD. The left parieto-occipital junction, however, was more strongly activated for guilt and less strongly activated for indignation in the MDD group. This region shows activation in response to socially relevant stimuli in general and may represent spatial and sensory aspects of

mental imagery [18] during the task. One possibility is therefore that individuals with MDD used more vivid mental imagery in the guilt condition and less in the indignation condition compared to the control group. This was, however, not reflected in their self-ratings but could have occurred implicitly.

Online-Only Tables

eTable 1 Ratings and response times for guilt and indignation trials

	Control mean ±SD	Remitted MDD mean ±SD	t-values	p-values
Frequency (%)				
guilt (self-agency)	27.9±10.8	30.2±10.5	.75	.46
indignation (other-agency)	32.9±14.9	38.3±14.4	1.27	.21
Rated unpleasantness				
guilt trials	4.6±.8	4.2±.7	-1.77	.08
indignation trials	5.1±1.1	4.6±.8	-1.72	.09
Response times (ms)				
guilt trials	2260±556	2259±452	-1.68	.99
indignation trials	2231±593	2204±526	-.01	.87
Number of possible consequences				
guilt trials	4.1±1.1	3.6±.8	-1.73	.09
indignation trials	4.3±1.2	3.8±.9	-1.52	.14
Intensity of visualization of described behaviour				
guilt trials	3.4±1.2	3.4±1.0	.28	.78
indignation trials	3.6±1.3	3.5±1.0	-.19	.85
Degree of autobiographical episodic retrieval				
guilt trials	2.4±1.0	2.7±1.2	.85	.40
indignation trials	2.4±1.0	2.6±.9	.88	.38
Social behavioural detail described by stimuli				
guilt trials	4.4±1.0	4.1±1.0	-1.22	.23
indignation trials	4.8±1.0	4.4±1.1	-1.50	.14
Difference of reference to self vs. best friend				
guilt trials	.1±.4	.3±.4	-1.62	.11
indignation trials	.2±.4	.3±.4	-.62	.54
IPG-67 Self-hate score	24.1±5.7	36.3±11.1	-4.8	.00003*

There were no between-group differences on any of the above measures at p=.05, two-sided (MDD group: N=25, control group: N=22). Ratings were obtained on 7-step visual analogue Likert scales (range 1 to 7, see details in Online-Only methods). Data for one MDD participant for the self- and best friend-reference measure were missing. Ratings for guilt and indignation trials as well as IPG-67 self-hate were also reported in a separate paper on the neuropsychological results of this study[19].

eTable 2 Between-group BOLD effect comparisons

Group	Contrast	Hem.	Region	BA	MNI X	MNI Y	MNI Z	t-value	FWE-corr. p-value
Control > MDD	guilt vs. indignation	R	posterior insula/superior temporal	21	44	-6	-8	4.1	.04 [c]
		L	parieto-occipital junction	39	-44	-64	36	4.4	.001[c]
MDD > control	guilt vs. indignation	-	no significant regions	-					
Control > MDD	indignation vs. guilt	-	no significant regions	-					
MDD > control	indignation vs. guilt	R	posterior insula/superior temporal	21	44	-6	-8	4.1	.04 [c]
		L	parieto-occipital junction	39	-44	-64	36	4.4	.001[c]

For ***guilt vs. indignation***, regions are reported that survived inclusive masking with ***guilt vs. fixation*** and for ***indignation vs. guilt***, regions surviving inclusive masking with ***indignation vs. fixation.*** c=cluster-based FWE-correction. There were no significant effects in our tier 1 or tier 2 ROIs, all reported areas survived FWE-correction over the whole brain. Control: N=22, remitted MDD: N=25.

eTable 3 Within-group standard BOLD analyses for guilt vs. indignation and indignation vs. guilt

Group	Contrast	Hemi-sphere	Region	BA	MNI coordinates			t-value	FWE-corr. p-value
					X	Y	Z		
Control	guilt vs. indignation	R	anterior middle temporal gyrus	21	52	10	-28	5.39	.02[1]
		R&L	dorsal anterior cingulate~	24	0	30	16	4.73	.000[1c]
		R	dorsal paracingulate~	32	10	14	48	4.43	.03^[c]
		R	cuneus	7	12	-72	32	4.15	.000^[c]
		L	superior anterior temporal lobe	22	-54	2	-14	6.12	.048[1]
		L	precentral gyrus	4	-38	-18	42	5.54	.000^[c]
		L	posterior superior temporal gyrus	39	-42	-56	18	4.46	.000[1c]
MDD	guilt vs. indignation		no significant regions						
Control	indignation vs. guilt		no significant regions						
MDD	indignation vs. guilt		no significant regions						

For ***guilt vs. indignation***, regions are reported that survived inclusive masking with ***guilt vs. fixation*** and for ***indignation vs. guilt***, regions surviving inclusive masking with ***indignation vs. fixation***. Regions marked~ are from analysis including N=21 controls. Regions marked [1] survived FWE-correction over a priori Tier 1 regions, regions marked [2] survived FWE-correction over a priori Tier 2 regions, and regions marked ^ survived FWE-correction over the whole brain. Regions marked [c]= cluster-level correction. Control: N=22, remitted MDD: N=25.

eTable 4 Between-group right superior ATL seed PPI effects

Group	Contrast	Hemisphere	Region	BA	MNI coordinates			t-value	FWE-corrected p-value
					X	Y	Z		
Control > MDD	guilt vs. indignation	L	subgenual cingulate/septal region	25	-6	22	0	4.67	.001[2]
		R	hippocampus	-	28	-16	-14	4.44	.03[1]
		R	lateral hypothalamus	-	12	-2	-12	3.67	.05[1]
		L	medial frontopolar cortex~	10	-2	66	20	3.97	.05[1c]
MDD > control	guilt vs. indignation		no significant regions						
Control > MDD	indignation vs. guilt		no significant regions						
MDD > control	indignation vs. guilt	L	subgenual cingulate/septal region	25	-6	22	0	4.67	.001[2]
		R	hippocampus	-	28	-16	-14	4.44	.03[1]
		R	lateral hypothalamus	-	12	-2	-12	3.67	.05[1]
		L	medial frontopolar cortex~	10	-2	66	20	3.97	.05[1c]

For ***guilt vs. indignation***, regions are reported that survived inclusive masking with ***guilt vs. fixation*** and for ***indignation vs. guilt***, regions surviving inclusive masking with ***indignation vs. fixation***. Regions marked~ are from analysis including N=21 controls. Regions marked [1] survived FWE-correction over a priori Tier 1 regions, regions marked [2] survived FWE-correction over a priori Tier 2 regions, regions marked c=cluster-based significance.

eTable 5 Within-group right superior ATL seed PPI effects

Group	Contrast	Hemi-sphere	Region	BA	MNI coordinates			t-value	FWE-corrected p value
					X	Y	Z		
control	guilt vs. indignation	L	subgenual cingulate/septal region	25	-6	22	0	4.67	.001[2]
		R	Hippocampus	-	30	-18	-14	5.03	.03[1]
		R	lateral hypothalamus	-	12	-2	-12	4.69	.01[1]
		L	amygdala	-	-24	-4	-22	4.14	.02[1]
		L	occipital pole	18	-16	-88	-14	4.59	.0001^[c]
		R	occipital pole	18	26	-84	-8	4.25	.0001^[c]
		R	dorsomedial paracingulate cortex~	32	2	38	28	4.34	.01^[c]
MDD	guilt vs. indignation		no significant effects	-	-	-	-	-	-
control	indignation vs. guilt		no significant effects	-	-	-	-	-	-
MDD	indignation vs. guilt		no significant effects	-	-	-	-	-	-

For ***guilt vs. indignation***, regions are reported that survived inclusive masking with ***guilt vs. fixation*** and for ***indignation vs. guilt***, regions surviving inclusive masking with ***indignation vs. fixation***. Regions marked [1] survived FWE-correction over a priori Tier 1 regions, regions marked [2] survived FWE-correction over a priori Tier 2 regions, and regions marked ^ survived FWE-correction over the whole brain, regions marked with [c] survived cluster-based correction. Control: N=22, remitted MDD: N=25, apart from regions marked ~=control: N=21 participants.

eTable 6 Individual between-subject differences in BOLD associated with guilt-proneness irrespective of group

Contrast	Hemisphere	Region	BA	MNI coordinates			t-value	FWE corr. p-value
				X	Y	Z		
Guilt vs. indignation	L	subgenual cingulate/septal region	25	-4	16	-6	4.63	.04[2]
	L	caudate body	-	-14	-6	20	3.13	.03[1]
	R	supragenual anterior cingulate cortex~	32	20	42	8	4.6	.03[1]

All regions are reported that survived inclusive masking with ***guilt vs. fixation,*** surviving an uncorrected threshold of p=.01, cluster equal to or greater than 4 voxels, and FWE-correction at p=.05, over a priori ROIs ([1]=Tier 1 ROIs, [2]=Tier 2 ROIs). Standard BOLD analysis was carried out for ***guilt vs. indignation,*** entering all subjects into a one-sample t-test. Covariates were included: individual percentages of guilt experienced across all self-agency trials as a covariate of interest, percentages of indignation/anger towards others experienced across all other-agency trials, and group (control or remitted MDD) were entered as covariates of no interest such that effects of guilt-proneness could be studied irrespective of group membership or indignation-proneness. Regions marked ~= N=21 control, other regions: N=22 control, all regions: N=25 remitted MDD participants.

eTable 7 Clinical characteristics of remitted MDD group (N=25)

Past MDD subtype	
With melancholic features	14/25
With melancholic & psychotic features	1/25
With atypical features	1/25
No specific subtype	9/25
Number of previous MDEs	
1	14/25
2	7/25
3	4/25
Last MDE details	
Average length of MDE (months)	16.6±19.1 (range: 3-96)
Average time in remission (months)	21.4±16.2 (range:12-84)
Severe*	22/25
Moderate*	2/25
Antidepressant medication at time of study	
SSRI/SNRI antidepressant	9/25
None	16/25
Previous medication in subgroup with no medication	
SSRI/SNRI antidepressant	10/16
SNRI and tricyclic combination	1/16
No antidepressant medication	5/16
Previous psychotherapy	6/25
Life-time axis-I co-morbidity*	
Anorexia nervosa	3/25
Anorexia nervosa, binge-eating subtype	1/25
Anorexia nervosa and bulimia nervosa	1/25
Post-traumatic stress disorder	1/25
No life-time co-morbidity	19/25
Family history	
First degree relative with MDD (diagnosed)	14/25
First degree relative with MDD (questionable)	4/25
Distant relative MDD	1/25
No family member with history of MDD	6/25

*According to ICD-10 criteria, ** All co-morbid disorders were fully remitted at time of study and none of the co-morbid disorders was a likely primary cause of the depressive episodes. SSRI=selective serotonin reuptake inhibitor, SNRI=serotonin norepinephrine reuptake inhibitor. MDD subtype classification was based on adapting the SCID-I for DSMIV-TR to allow lifetime assessment of subtypes. All medication-free participants had stopped medication well before the required washout phase. A similar table may be reported in other papers presenting secondary data analyses.

eTable 8 Exclusion of volunteers following phone pre-screening interview

Reason for exclusion	N
Control and Remitted MDD groups	
Substance or alcohol abuse	9
MRI contraindications	5
Other psychiatric disorders than MDD	18
Severe developmental disorders	1
General medical condition	7
Family history of MDD/bipolar/schizophrenia (Control) or bipolar/schizophrenia (MDD)	9
Current antidepressant (Control) or other centrally active medications (MDD)	3
Left-handed	2
Non-native English speaker	9
Remitted MDD group only	
Not meeting full screening criteria for MDE	5
Not remitted for 12 months	16
Fulfilling criteria for current MDE	8
Total excluded after phone pre-screening	***92***

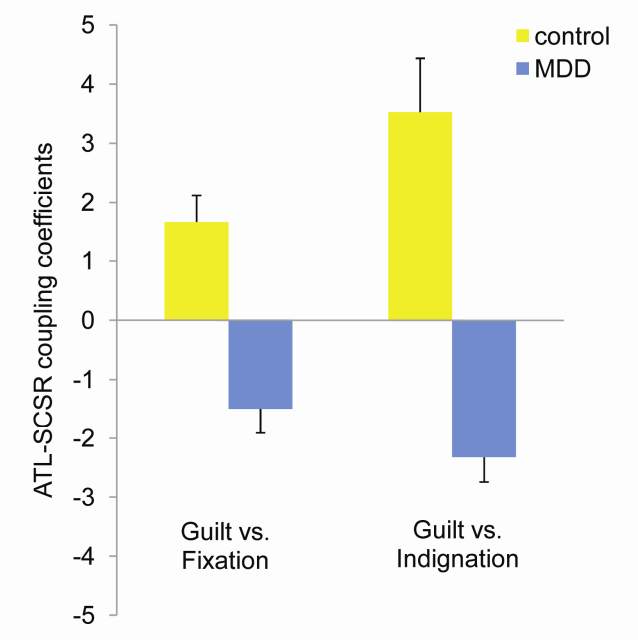
In total, 171 people participated in the phone pre-screening interview, N=79 passed this screening with 36 in the remitted MDD and 43 in the control group and were invited for the first study day. Of these, 33 individuals pre-screened as remitted MDD and 30 pre-screened as control participants were reachable, able and willing to be seen on the first study day after reading the participant information sheet sent to them. After the first day of the study, 5/33 individuals from the remitted MDD group were excluded (N=1 fulfilled criteria for current MDD, N=2 showed residual symptoms of post-traumatic stress disorder, N=1 had a relapse and developed an MD episode after the first study day before being scheduled for MRI), the remaining N=28 participants confirmed as remitted MDD underwent MRI. MRI data from 25/28 scanned participants from the MDD group could be included in the analysis (N=2 were excluded because of head movement greater than 4 mm, 1 because of selecting more than one moral sentiment in more than 5% of trials). All 30 participants seen on the first study day who had fulfilled phone pre-screening criteria for the healthy control group were confirmed as fulfilling inclusion and exclusion criteria on clinical assessments and were invited for MRI scanning, however, 1 was not scanned because not being reachable following the first study session, leaving 29 that were scanned. Data from 22/29 scanned control participants could be included in the final analysis (data from N=1 was excluded because of selection of more than one feeling on more than 5% of trials, N=1 due to abnormalities of small vessels on the MRI scan, N=1 due to head movement greater than 4 mm, N=2 because of signal drop-outs in main ROIs: ventral frontal cortex and ATL, N=1 because of fewer than 4 guilt trials on one of the runs, and N=1 for age-matching between the final control and MDD groups). A similar table may be reported in other papers presenting secondary data analyses.

eTable 9 Group comparison on demographic and basic clinical variables

	Control	Remitted MDD	Test statistic	p-value
Age	22.8±3.3	25.6±7.5	t=-1.71	.10
Education (years)	15.6±1.6	16.2±2.1	t=-1.04	.31
Gender	18 Female	20 Female	CC=.02	.87
MADRS	.3±.7	1.1±1.7	U=207.5	.06
GAF	89.6±4.9	83.9±6.9	U=143.0	.002*

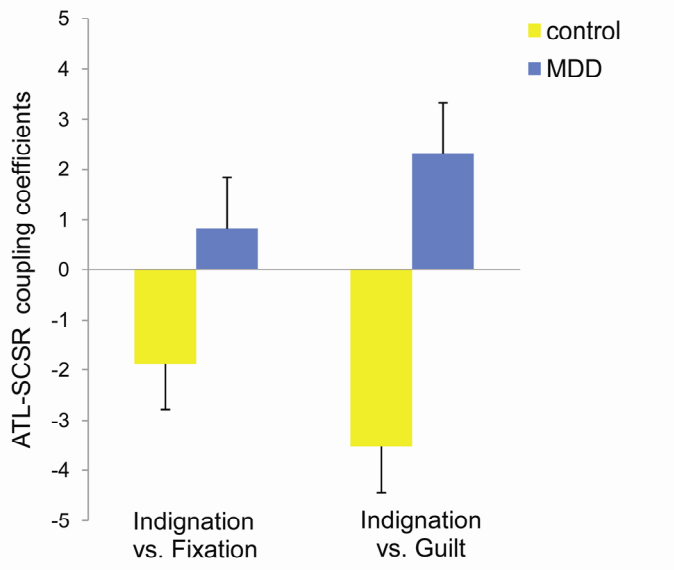
CC=contingency coefficient, *=significant at p=.05 threshold, 2-tailed, control: N=22, remitted MDD: N=25, U=Mann-Whitney-U. A similar table may be reported in other papers presenting secondary data analyses.

Online-Only Figures



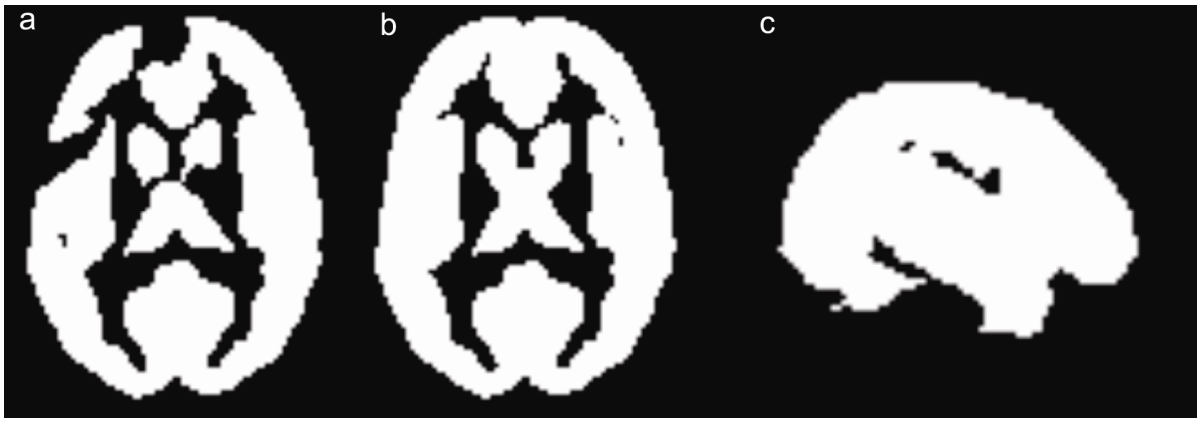
eFigure 1

Peak-voxel regression coefficients for ATL-SCSR PPI for guilt vs. indignation with standard error bars (control N=22, remitted MDD N=25).



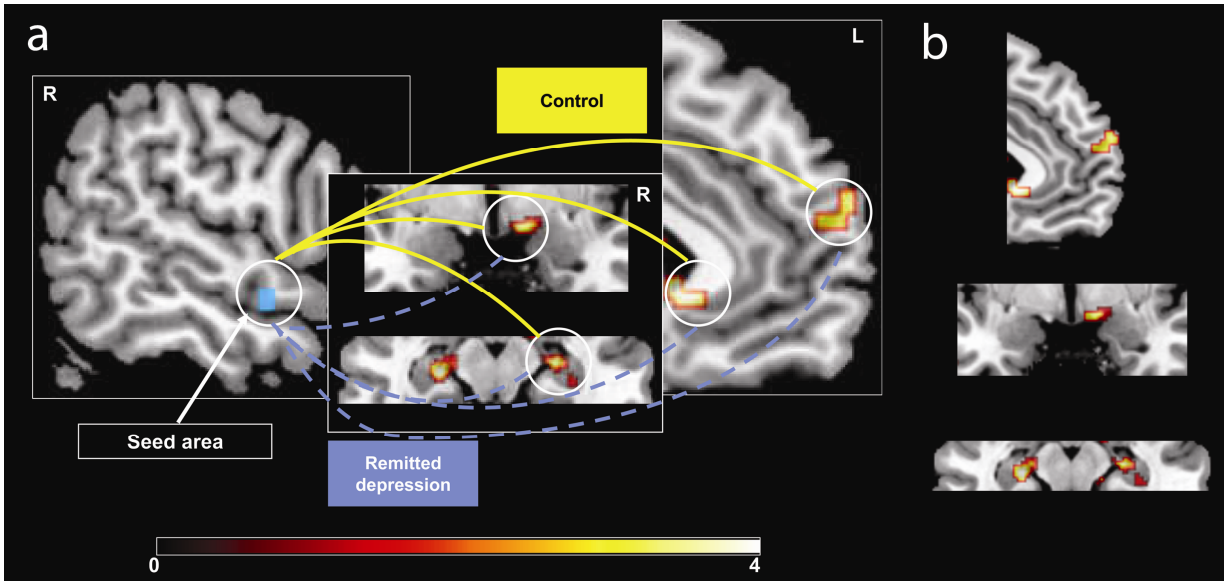
eFigure 2

Peak-voxel regression coefficients for ATL-SCSR PPI for indignation vs. guilt with standard error bars (control N=22, remitted MDD N=25).



eFigure 3

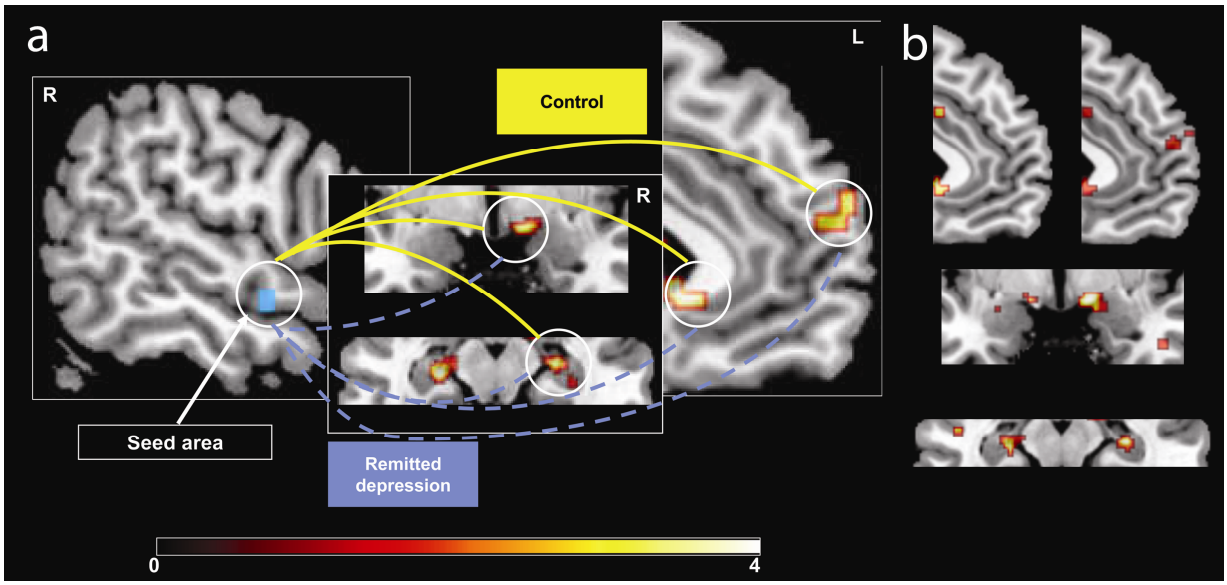
Panel a) shows an axial slice at z=14 through the implicit mask generated by SPM for the group-level analysis including N=22 controls and N=25 individuals with remitted MDD: The frontopolar cortex drop-out was from z= 23 to z=9. Analyses were performed and statistics were used from all regions that did not exhibit signal dropout. Panel b) shows an axial slice at z=14 through the implicit mask for analysis including N=21 controls and N=25 remitted MDD. There was full coverage of the dorsolateral and dorsomedial PFC including the frontopolar cortex. The border for ventral coverage of the most anterior portion of ventromedial PFC was z= -19. Coverage of posterior orbitofrontal cortex, a region prone to signal drop-out was adequate for regions superior to z= -12, the subgenual cingulate cortex from z= -14, and coverage of the most dorsal slice of the brain was up to z=59. Analyses were performed and statistics used from this analysis for dorsomedial, dorsolateral and frontopolar regions. Panel c) shows a sagittal slice at x=48 trough the implicit mask generated by SPM (N=22 control and N=25 MDD). Coverage of the superior ATLs was complete posterior to y=+11 and medial to +56. Full coverage of the middle ATLs extended posteriorly up to y=-5. The inferior ATLs, not of main interest in this study because of their role in general conceptual knowledge [20] rather than selectivity for social concepts [16], showed signal drop-outs as usual on Gradient Echo EPI and could only have been reliably covered by using optimized Spin Echo sequences [21] which would not have been suitable because their long repetition time would not have permitted the rapid event-related design necessary for this study.



eFigure 4

Panel a) displays a copy of Fig 1 of the main manuscript: regions showing decreased coupling with the right superior ATL during the experience of ***guilt vs. indignation*** in individuals with remitted MDD compared with

healthy controls including the lateral hypothalamus, hippocampus, medial frontopolar cortex and a subgenual cingulate/septal region. Cropped whole brain images were displayed at an uncorrected threshold of p=.005 (extent threshold of 4 voxels). All depicted regions survived FWE-correction over a priori ROIs at p=.05 in separate analyses. Panel b) shows the results of the same analysis, but using mean unpleasantness ratings for guilt and indignation conditions for each participant as a covariate of no interest. As is evident, the covariance analysis results in the same clusters as showing PPI effects irrespective of individual differences in perceived unpleasantness of stimuli.



eFigure 5

Panel a) displays a copy of Fig 1 of the main manuscript: regions showing decreased coupling with the right superior ATL during the experience of ***guilt vs. indignation*** in individuals with remitted MDD compared with healthy controls including the lateral hypothalamus, hippocampus, medial frontopolar cortex and a subgenual cingulate/septal region. Cropped whole brain images were displayed at an uncorrected threshold of p=.005 (extent threshold of 4 voxels). All depicted regions survived FWE-correction over a priori ROIs at p=.05 in separate analyses. Panel b) shows the PPI results for the control group (N=21) for ***guilt vs. indignation*** inclusively masked with ***fixation vs. indignation*** both at the same threshold as in panel a). This comparison thus reveals those regions in the control group in which there is lower coupling in the ***indignation*** condition relative to ***fixation*** and ***guilt***. When comparing the results in panel a) and b), one can see that a decrease in ATL-coupling during the experience of ***indignation*** in the control group contributes to group differences in addition to the demonstrated increase in ATL-coupling in the ***guilt*** condition (eTable4, Table 1). Apart from the medial frontopolar region, the regional distribution of clusters was highly overlapping between both analyses (a & b).

Online-Only References

1. DSM-IV. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition - Text Revision (DSMIV-TR)*; American Psychiatric Association 2000.
2. Janca A, Ustun T, van Drimmelen J, Dittmann V, Isaac M. ICD-10 Symptom Checklist for Mental Disorders: Version 1.1. Geneva, Division of Mental Health, World Health Organization.; 1994.
3. Montogomery SA, Asberg M. A new depression scale designed to be sensitive to change. *British Journal of Psychiatry*. 1979;134:382-389.
4. Lecrubier Y, Sheehan DV, Weiller E, Amorim P, Bonora I, Sheehan KH, Janavs J, Dunbar GC. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: Reliability and validity according to the CIDI. *European Psychiatry.* 1997;12(5):224-231.
5. Green S, Lambon Ralph MA, Moll J, Stamatakis EA, Grafman J, Zahn R. Selective functional integration between anterior temporal and distinct fronto-mesolimbic regions during guilt and indignation. *NeuroImage.* 2010;52(4):1720-1726.
6. Zahn R, Moll J, Paiva M, Garrido G, Kruger F, Huey ED, Grafman J. The Neural basis of Human Social Values: Evidence from fMRI. *Cerebral Cortex.* 2009;19:276-283.
7. Piefke M, Weiss PH, Markowitsch HJ, Fink GR. Gender differences in the functional neuroanatomy of emotional episodic autobiographical memory. *Human Brain Mapping.* Apr 2005;24(4):313-324.
8. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage.* 2002;15(1):273-289.
9. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage.* Jul 2003;19(3):1233-1239.
10. Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas ES, Rainey L, Kochunov PV, Nickerson D, Mikiten SA, Fox PT. Automated Talairach Atlas labels for functional brain mapping. *Human Brain Mapping.* 2000;10(3):120-131.
11. Moll J, de Oliveira-Souza R, Garrido GG, Bramati IE, Caparelli-Daquer EMA, Paiva MLMF, Zahn R, Grafman J. The self as a moral agent: linking the neural bases of social agency and moral sensitivity. *Social Neuroscience.* 2007;2:336-352.
12. Moll J, Krueger F, Zahn R, Pardini M, de Oliveira-Souza R, Grafman J. Human fronto-mesolimbic networks guide decisions about charitable donation. *Proceedings of the National Academy of Sciences of the United States of America.* Oct 17 2006;103(42):15623-15628.
13. Zahn R, Moll J, Iyengar V, Huey ED, Tierney M, Krueger F, Grafman J. Social conceptual impairments in frontotemporal lobar degeneration with right anterior temporal hypometabolism. *Brain.* 2009;132:604-616.
14. Krueger F, McCabe K, Moll J, Kriegeskorte N, Zahn R, Strenziok M, Heinecke A, Grafman J. Neural correlates of trust. *Proceedings of the National Academy of Sciences of the United States of America.* 2007;104(50):20084-20089.
15. Zahn R, de Oliveira-Souza R, Bramati I, Garrido G, Moll J. Subgenual cingulate activity reflects individual differences in empathic concern. *Neuroscience Letters.* 2009;457(2):107-110.
16. Zahn R, Moll J, Krueger F, Huey ED, Garrido G, Grafman J. Social concepts are represented in the superior anterior temporal cortex. *Proceedings of the National Academy of Sciences.* 2007;104(15):6430-6435.
17. Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha IS, Bryson H, de Girolamo G, de Graaf R, Demyttenaere K, Gasquet I, Haro JM, Katz SJ, Kessler RC, Kovess V, Lepine JR, Ormel J, Polidori G, Russo LJ, Vilagut G, Almansa J, Arbabzadeh-Bouchez S, Autonell J, Bernal M, Buist-Bouwman MA, Codony M, Domingo-Salvany A, Ferrer M, Joo SS, Martinez-Alonso M, Matschinger H, Mazzi F, Morgan Z, Morosini R, Palacin C, Romera B, Taub N, Vollebergh WAM, Investigators E-M,

. Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiatrica Scandinavica.* 2004;109:21-27.

18. Moll J, de Oliveira-Souza R, Zahn R. The neural basis of moral cognition - Sentiments, concepts, and values. *Year in Cognitive Neuroscience 2008.* 2008;1124:161-180.

19. Green S, Moll J, Deakin JF, Hulleman J, Zahn R. Proneness to decreased negative emotions in major depressive disorder when blaming others rather than oneself. submitted.

20. Visser M, Embleton KV, Jefferies E, Parker GJ, Lambon Ralph MA. The inferior, anterior temporal lobes and semantic memory clarified: Novel evidence from distortion-corrected fMRI. *Neuropsychologia.* 2010;48(6):1689-1696.

21. Embleton KV, Haroon HA, Morris DM, Ralph MAL, Parker GJM. Distortion Correction for Diffusion-Weighted MRI Tractography and fMRI in the Temporal Lobes. *Human Brain Mapping.* Oct 2010;31(10):1570-1587.

22. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition. (SCID-I/P)* New York: Biometrics Research, New York State Psychiatric Institute; 2002.

23. Parker G, Hadzipavlovic D, Austin MP, Mitchell P, Wilhelm K, Hickie I, Boyce P, Eyers K. Sub-Typing Depression, .1. Is Psychomotor Disturbance Necessary and Sufficient to the Definition of Melancholia. *Psychological Medicine.* Jul 1995;25(4):815-823.

24. Ebert D. Alterations of Drive in Differential-Diagnosis of Mild Depressive-Disorders - Evidence for the Spectrum Concept of Endogenomorphic Affective Psychosis. *Psychopathology.* Jan-Feb 1992;25(1):23-28.

Appendix

Phone pre-screening interview

- **Instructions for Interviewer are marked in bold.**

Oral consent to be read first:

"I would like to do a short phone interview with you which will take around 15 minutes. This is necessary to see whether some conditions rule out that we can include you into the study. You will be asked questions about psychiatric, neurological and medical symptoms, treatments, learning problems and whether such symptoms have occurred in your family. I will also ask about substance or alcohol abuse. Things which are an obstacle to participate in MRI studies such as possible pregnancy or metallic objects will also be asked. Results of these questions will not be stored, but we ask your permission to store your contact information and whether you passed the screening for the study group in an electronic database which is protected by a password and can only be accessed by the investigators."

Question	Response		Comments
Do you agree to this interview?	yes	no	**If no => Exclusion**
How many years of education do you have?			**towards the end of the study, controls will be selected to be age- and education- matched to the patient population**
What is your date of birth ?			**If < 18 => Exclusion, If > 65, exclusion for study**
Are you right-handed?	yes	no	**If no => Exclusion**
Is English your first language?	yes	no	**If no => Exclusion**
Are you currently taking any medications?	yes	no	
If yes -> What medications?			
Have you ever, at any time, taken anti-depressant or anti-psychotic medications (such as Prozac, Zoloft, Zyprexa, Haldol)?	yes	no	**If yes => Exclusion as Healthy control**
Have you ever been diagnosed with or treated for any psychiatric or psychological problem (for example: Depression, Bipolar or manic-depressive, Anxiety, Posttraumatic Stress, Eating, Borderline Personality, Obsessive-Compulsive, Psychotic or Schizophrenic disorders, Attention-Deficit-Disorder) ?	yes	no	**If yes => Exclusion as Healthy control. Anxiety Disorders & ADHD allowed in MDD groups if not prominent.**

Have you ever been diagnosed with or treated for any neurological problem (palsy, gaze problems, gait problems, motor coordination, epilepsy, stroke, parkinson's)?	yes	no	**If yes => Exclusion**
Have you ever had a drug or alcohol problem?	yes	no	**If yes => Exclusion**
Have any of your first degree relatives (parents, siblings or children) ever been treated for or diagnosed with psychosis, schizophrenia, depression, bipolar disorder or manic depression?	yes	no	**If yes => Exclusion as Healthy control**
Have you ever had any significant physical health problems, for example heart, lung problems, diabetes, hypertension, diabetes, arterial diseases, thyroid function problems, liver, kidney disorders, rheumatoid disorders, infectious diseases or anything else?	yes	no	**If yes => check w. PI whether exclusion criterion**
Have you ever had any learning disabilities?	yes	no	**If yes => Exclusion**
Do you have hearing problems or problems with vision?	yes	no	**If yes => Exclusion if canot be corrected for experiment**

Adapted MINI screening questions for all patient groups [4]

Have you ever been consistently depressed or down, most of the day, nearly every day, for at least 2 weeks?	yes	no	**If yes => Exclusion as Healthy control, check eligibility for MDD groups**
In the last 12 months have you ever had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions?	yes	no	**If yes => Explore further**
In the last 12 months have you taken any drugs more than once: for example stimulants, amphetamines, diet pills, cocaine, morphine, LSD, "mushrooms","ecstasy", cannabis ("hash"), tranquilizers, steroids, sleep pills or pain killers?	yes	no	**If yes => Explore further**
In the last 12 months have you been intoxicated, high, or hungover from alcohol or drugs when you had other responsibilities (work, school, home) or did you have legal problems, problems with other people or accidents because of this?	yes	no	***If yes or questionable => Exclusion***

Screening questions for all patient groups (major psychiatric disorders)

Have you ever had a phase of at least 2 weeks in your life where you needed only a few hours (for example 3 h) of sleep and were still totally alert and very active the whole day, where you were very enthusiastic and did things you usually wouldn't do?	yes	no	**If yes => Exclusion**
Have you ever been traumatized in a way, that you feared your life was in danger or were you sexually assaulted (Please			**If yes => Exclusion**

just say “yes” or “no”, no details will be asked nor recorded)?	yes	no	
Do you experience frequent states of tension and use self-injuries such as cutting or burning to reduce tension?	yes	no	**If yes => Exclusion**
Do you get very tense or anxious, when your personal things (i.e. on your desk) are not symmetrically arranged, when you can’t wash your hands, after you have touched a door knob, when you can’t perform certain daily activities according to a fixed and detailed routine (i.e. washing, certain professional or household activities)? (If yes: Does this interfere with your professional or personal life?)	yes	no	**If yes => Exclusion**
Have you ever heard voices with no person or audio-device as a source?	yes	no	**If yes => Exclusion**
Have you ever lost control of your body movements or your thoughts and felt controlled by an external power?	yes	no	**If yes => Exclusion**
Have you experienced unusual signs referring specifically to you and indicating great danger, for example by a group or person threatening your life?	yes	no	**If yes => Exclusion**

Questions asked for remitted MDD group, partly taken from the MINI-screening questionnaire [4]

When was your last depressive phase?			
When did you start to feel well again?			**> 12 months healthy to be included**
Do you now feel as well as before your first depressive phase and do you feel completely healthy?	yes	no	**only included if yes**
In your most severe depressive phase, have you been consistently depressed or down, most of the day, nearly every day, for at least 2 months?	yes	no	**only included if yes**
During the most severe period of that depressive episode, did you have a general loss of drive and energy, where your activities were either slowed down or only possible against a huge inner resistance?	yes	no	**only included if yes**
During the most severe period of that depressive episode, did you lose almost completely your ability to enjoy nearly everything?	yes	no	**only included if yes**

Eligibility for MRI study (adapted from NIH/NINDS consent form used in [16])

For women: Are you absolutely sure that you are not pregnant?	yes	no	**exclusion for MRI if no**
Do you have dental implants such as fillings or crowns (not made from gold)?	yes	no	**potential exclusion for MRI if yes because of reduced image quality**

Do you have permanent eyeliner or other permanent make-up?	yes	no	**potential exclusion for MRI if yes because of reduced image quality**
Do you have loose dental implants such as fillings or crowns which canot be removed before scanning?	yes	no	**absolute exclusion for MRI if yes**
Do you have any implanted electrical devices? (pacemaker, brain stimulator, ear implants, implanted delivery pumps)	yes	no	**exclusion for MRI if yes**
Could you have any metal in your body?(metal clips on the wall of a large artery, metallic prostheses including metal pins and rods, heart valves, shrapnel fragments)	yes	no	**exclusion for MRI if yes**
Have you ever worked as a welder or metal worker? (this can lead to small metal fragments in the eye which you may be unaware of)	yes	no	**exclusion for MRI if yes**
Do you get anxious in confined spaces?	yes	no	**exclusion for MRI if yes**
Do you require a hearing aid?	yes	no	**exclusion for MRI if yes**

Interview is stopped as soon as exclusion criterion is detected, the interviewer apologizes for not being able to include the person and thanks again for the willingness to participate. If necessary, one can explain that it is important for research studies to focus on specific types of depression, because otherwise it is difficult to find significant results if patients with different types of depression or other problems are mixed together.

If person meets all inclusion/exclusion criteria for one of the study groups (healthy control – remitted MDD), contact information and study group are stored in password protected excel sheet. The Participant Information Sheet (PIS) and Consent for the respective study is sent to the person after screening and an appointment for day 1 is scheduled with at least 24 h time after the person has received the PIS and signed the consent. This sheet is reviewed after the phone interview and then shredded.

Comment: The screening questions for major psychiatric disorders are based on clinical experience as providing high sensitivity and specificity for bipolar disorder, schizophrenia, OCD, PTSD and Borderline Personality Disorder. The screening questions for inclusion into MDD group were aimed at recruiting participants with phasic and severe forms of depression with good intermittent recovery and were inspired by the melancholic subtype questions of the MINI[4]/SCID[22] as well as work on the importance of "inhibition of drive" in identifying melancholia [23, 24].