Supplementary Information

Supplementary Data

Details of participants

Inclusion criteria for currently depressed participants were an acute episode of unipolar major depression and a score≥20 on the MADRS. Patients in remission were included if they had experienced at least one past episode of unipolar major depression and scored≤10 on the MA DRS. Thirty-seven of the currently depressed patients (N=40) experienced recurrent depression and had been well for an average of 5 years prior to recruitment. Fifteen currently depressed patients were treatment naïve at the time of enrolment whereas 35 had experienced 1-3 previous episodes treated with antidepressants (mode = 1) and had been off treatment for an average of 4 years. All the remitted patients had been well for longer than a year, part from one participant well for 4 months. An estimation based on the time since last contact with services in 20 remitted depressed suggested a mean 6.1 years, SD=4.6 of remission. Nine remitted patients were medication naïve when recruited, whereas 13 had experienced one episode and 2 two episodes treated with antidepressants.

Vision was normal or corrected to normal for all participants. Recruitment was conducted in the University of Manchester by using websites, local advertisement, referrals from general practice and word of mouth. Structural images were examined by a consultant neuroradiologist to exclude any gross structural abnormality. Some of the remitted depressed participants and controls were recruited as part of a larger project investigating vulnerability factors for depression (NewMood study: http://www.medicine.manchester.ac.uk/psychiatry/newmood/). Findings from a larger group of both medicated and unmedicated remitted depressed patients (N=30) compared with a subset of controls (N=37) have recently been published (1).

Citalopram adherence in currently depressed patients

Plasma citalopram was measured at Oxford Brooks University (UK) by reverse-phase High Performance Liquid Chromatography with utilisation of fluorescence end-point detection. Citalopram was extracted from plasma via two-phase solvent to acid extraction procedure with utilisation of an internal standard to monitor extraction recovery. The intra and inter assay CVs were < 7% for a pool sample of 10ng/ml when a plasma volume of 0.5ml was extracted and stored at -80 ˚C in heparinized tubes until study completion.

Details of the indirect emotion processing task

The emotions were presented at 80% emotion intensity (taken from a series of morphed images between the full emotion and neutral) which provides the minimum intensity for full recognition (2). Each face was displayed for 3000ms, with an inter-stimulus interval of 500ms, giving each block a total length of 21 seconds. Seven blocks consisting of happy (H), sad (S) or fearful (F) faces were presented interspersed by neutral (N) faces and followed by a rest block (NHNSNFNR). This was repeated 3 times with the emotion blocks presented in a pseudo-random order within each repeated series (High Pass Filter cut off=300s). Total task length was therefore 8 minutes and 24 seconds.

Details of fMRI data acquisition and statistical analyses

1) Data acquisition

Each volume consisted of 29 contiguous axial slices with a slice thickness of 4.5mm, a slice gap of 0.5mm and in-plane resolution, 3.5 x 3.5mm. Images were motion corrected, spatially normalised to standard Montreal Neurological Institute (MNI) space and smoothed using a Gaussian kernel filter of 10mm to allow for inter-subject differences.

2) Statistical analysis

First level contrasts were entered into an analysis of variance (ANOVA) using the full factorial model implemented in SPM5, with emotion (happy, sad, fearful) as the within-subjects factor and group (healthy controls, remitted depressed and currently depressed) as the between-subject factor to investigate the main effects of group and emotion, and the group x emotion interaction. Emotion x group interactions were further investigated by using two sample t-tests in SPM. In the longitudinal study, comprising currently depressed and a subgroup of healthy controls receiving two MRI scans 8 weeks apart, the primary analysis was based on the emotions showing significant results from the cross-sectional study using t-tests to compare the change between visits (V1-V2) both between groups and for currently depressed and healthy controls separately.

Details of correlational analyses

Potential effects of clinical variables were explored in SPM with correlation analyses by using one sample t-tests. Each variable of interest was individually entered as a covariate in the model. We investigated the possibility of haemodynamic responses to facial emotions being affected by altered responses to neutral faces by comparing neutral faces with rest data. Interrogation of the whole brain at $p_{unc}<0.001$ revealed correlations in para-hippocampal gyrus - a region known to be involved in emotional processing (2). Signal intensity at V1 positively correlated with CAS score in the right parahippocampal gyrus for fear-neutral faces (R: 21 -25 -20; Z=3.56; p=0.00019). The change in sad-neutral signal intensity between V1 and V2 correlated negatively with % change in MADRS score in bilateral

parahippocampal gyrus (R: 21 -32 -10; Z=3.36; p=0.00039; L: -25 -25 -15; Z=3.68; p=0.00012), i.e. a greater improvement in depression was associated with a greater decrease in parahippocampal gyrus activation. There were no significant correlations between citalopram levels and neural activity in any of the other comparisons.

Further longitudinal analyses

Including the 2 participants without citalopram in their serum at the second scan (N=25) did not substantially alter the findings (R: 32, -4, -20; Z=2.71; p_{FWE}=0.042; L: -28 0 -25; Z=2.55; p_{FWE}=0.063). Similarly, amygdala responses to sad faces in the whole group of returners (N=30) showed increased responses at V2 in the right amygdala (32, -4, -20; Z=2.87; p_{FWE}=0.028) with a trend in the left amygdala (-28 0 -25; Z=2.61; p_{FWE}=0.054).

References

1. **Thomas, E. J., Elliott, R., McKie, S., Arnone, D., Downey, D., Juhasz, G., Deakin, J.F., Anderson, I. M., 2011. Interaction between a history of depression and rumination on neural response to emotional faces. Psychol Med. 2011 41(9):1845-55.**
2. Fu, C. H., Williams, S. C., Cleare, A. J., Brammer, M. J., Walsh, N. D., Kim, J., Andrew, C. M., Pich, E. M., Williams, P. M., Reed, L. J., Mitterschiffthaler, M. T., Suckling, J., Bullmore, E. T., 2004. Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. Archives of general psychiatry 61, 877-889.
3. Victor, T.A., Furey, M.L., Fromm, S.J., Ohman, A., Drevets, W.C., 2010. Relationship between amygdala responses to masked faces and mood state and treatment in major depressive disorder. Archives of general psychiatry 67, 1128-1138.

Supplementary Tables

Table S1: Blood oxygenation level dependent responses elicited bilaterally in occipital areas and fusiform gyrus by emotional faces (sad, fear and happy) in comparison with neutral faces in the analysis of all participants across all groups (Emotion-Neutral for all groups combined), whole brain $p < 0.001$ uncorrected (* = p(FWE)<0.05); k = number of voxels in cluster at p=0.001 uncorrected; as no up-sampling was performed at the spatial normalisation stage of the preprocessing the originally collected voxel size of 3.5x3.5x5mm (61.25mm^3) was used throughout all stages of the analysis:
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	Emotion-Neutral for all groups combined						
Region	**BA**	**Side**	**k**	**x**	**y**	**z**	**z-score**
Occipital Gyrus	19	L	410	-28	-91	0	8.23*
		R	441	32	-91	5	8.38*
Fusiform Gyrus	37	L	85	-42	-46	-20	6.54*
		R	58	42	-49	-15	7.61*
Middle Temporal Gyrus	21/37	L	14	-60	-56	5	3.58
		R	103	60	-56	5	5.42*
Amygdala	N/A	L	17	-21	-4	-15	4.18
		R	5	28	-7	-15	3.41
Frontal Eye Field	8/9	L	6	-53	14	30	3.47
		R	17	56	21	25	4.16

Tables S2-S6: Exploratory analyses were conducted at whole brain level to investigate blood oxygenation level dependent responses to face emotions and to each individual emotion (sad, fear and happy) in relation to neutral faces across groups. Only p<0.001uncorrected results are reported; k=number of voxels; as no up-sampling was performed at the spatial normalisation stage of the preprocessing the originally collected voxel size of 3.5x3.5x5mm (61.25mm^3) was used throughout all stages of the analysis:

Table S2: Emotion-Neutral x group, whole brain exploratory analyses

			Emotion-Neutral x group				
Region	**BA**	**Side**	**k**	**x**	**y**	**z**	**z-score**
Amygdala	N/A	R	8	18	4	-15	3.50
Fusiform Gyrus	19	L	3	-11	-77	-5	3.24
Anterior Cingulate	32	-	1	4	42	30	3.14
Precuneus	7	L	1	-11	-53	60	3.12

Table S3: Sad-Neutral x group, whole brain exploratory analyses

			Sad-Neutral x group				
Region	**BA**	**Side**	**k**	**x**	**y**	**z**	**z-score**
Fusiform/Lingual Gyrus	18/19	L	5	-21	-81	-15	3.66
		R	5	18	-88	-10	3.32
Precuneus	7	-	29	-4	-74	45	3.56
Middle Temporal Gyrus	21	R	5	56	-32	-10	3.39
Amygdala	N/A	R	7	35	-7	-20	3.30
Superior Temporal Gyrus	38	R	1	49	0	-15	3.12

Table S4: F-N x group, whole brain exploratory analyses

			Fear-Neutral x group					
Region	**BA**	**Side**	**k**	**x**	**y**	**z**	**z-score**	**Group Differences**
Precentral Gyrus	6	R	16	56	-7	30	3.83	Currently Depressed>Healthy Controls; Currently Depressed>Remitted Depressed
Insula	13	R	11	46	-4	-10	3.63	Currently Depressed>Remitted Depressed
Cuneus	19	R	9	28	-88	25	3.63	Healthy Controls>Currently Depressed; Remitted Depressed>Currently Depressed
Inferior Parietal Lobule	40	R	5	67	-42	30	3.34	Currently Depressed>Remitted Depressed
Fusiform/Lingual Gyrus	18	L	1	-11	-81	5	3.09	Healthy Controls>Currently Depressed
	19	R	9	32	-70	-10	3.63	Healthy Controls>Currently Depressed; Remitted Depressed>Currently Depressed

Table S5: H-N x group, whole brain exploratory analyses

			Happy-Neutral x group					
Region	**BA**	**Side**	**k**	**x**	**y**	**z**	**z-score**	**Group Differences**
Precuneus	7	L	12	-14	-53	50	3.57	Currently Depressed>Healthy Controls; Currently Depressed>Remitted Depressed

Dentate	n/a	R	5	18	-56	-30	3.33	Currently Depressed>Healthy Controls; Currently Depressed>Remitted Depressed

Table S6: N-R x group, whole brain exploratory analyses

	Neutral-Rest x group						
Region	**BA**	**Side**	**k**	**x**	**y**	**z**	**z-score**
Medial Frontal Gyrus	6	R	17	11	4	65	3.99

Effect seen in currently depressed only with all other emotions compared to rest showing the same effect in the medial frontal gyrus.

Table S7: Exploratory analyses were carried out to investigate the pattern of neural responses across groups with Sad-Neutral contrast. Only differences in blood oxygenation level dependent responses significant at p<0.001uncorrected are reported. k = number of voxels; As no up-sampling was performed at the spatial normalisation stage of the preprocessing the originally collected voxel size of 3.5x3.5x5mm (61.25mm^3) was used throughout all stages of the analysis:

Sad-Neutral, Currently Depressed>Remitted Depressed

Region	BA	Side	k	x	y	z	z-score
Amygdala	N/A	L	5	-35	0	-25	3.52
		R	16	35	-7	-20	3.89
Superior Temporal Gyrus	42	L	5	-63	0	15	3.70
	38	L	5	-42	4	-25	3.65
		R	6	49	0	-15	3.61
Insula	13	L	1	-39	-7	-5	3.11
		R	6	39	7	-5	3.40
Inferior Frontal Gyrus	47	R	7	28	18	-15	3.21

Sad-Neutral, Remitted Depressed>Currently Depressed

Region	BA	Side	k	x	y	z	z-score
Fusiform/Lingual Gyrus	18/19	L	38	-21	-81	-15	4.22
		R	24	18	-88	-10	3.88
Precuneus	7	-	90	-4	-74	45	4.14
Cuneus	18	L	10	-18	-88	25	3.44
Superior Temporal Gyrus	21/22	L	6	-60	-21	-5	3.35
		R	10	67	-42	5	3.68

Sad-Neutral, Currently Depressed>Healthy Controls

Region	BA	Side	x	y	z	z-score
No supra-threshold clusters						

Sad-Neutral, Healthy Controls>Currently Depressed

Region	BA	Side	k	x	y	z	z-score
Lingual Gyrus	17	R	17	14	-88	-5	3.66
Cuneus	19	L	12	-25	-84	30	3.62
	19	R	3	32	-88	10	3.29
Frontal Eye Field	8/9	L	30	-42	7	45	3.59
		R	1	39	-4	55	3.14
Middle Temporal Gyrus	21	R	7	56	-32	-10	3.54
	37	R	1	53	-70	0	3.24
Middle Frontal Gyrus	6	R	2	35	-7	60	3.36
Precuneus	7	L	1	-11	-74	45	3.15

Sad-Neutral, Remitted Depressed>Healthy Controls

Region	BA	Side	x	y	z	z-score
No supra-threshold clusters						

Sad-Neutral, Remitted Depressed>Healthy Controls

Region	BA	Side	k	x	y	z	z-score
Cuneus	17	R	13	11	-95	0	3.82
Precentral Gyrus	6	L	1	-42	-4	35	3.11