Supplemental Information

Medication load

A problem for all neuroimaging studies of bipolar disorder (BD) is the potential confounding effect of psychotropic medication, as it is difficult to recruit medication-free BD individuals into such studies (1). We wished to examine the potential impact of psychotropic medication upon BOLD signal in BD and major depressive disorder (MDD) individuals using an index of "medication load". This index reflects the number and dose of different medications for each individual: the greater the number and dose of the medication, the greater the medication load. This strategy has been employed in our previous neuroimaging studies in BD (1-3). For antidepressants and mood-stabilizers we converted each medication into low- or high-dose groupings. We coded as low-dose individuals on levels 1 and 2, and as high dose, individuals on levels 3 and 4 of these previous criteria (4). We added a no-dose subtype for those not taking these medications. We converted antipsychotics into chlorpromazine dose equivalents, and coded as 0, 1 or 2, for no medication, chlorpromazine equivalents dose equal or below, or above, mean effective daily dose (ED50) of chlorpromazine (5). Benzodiazepine dose was coded as 0, 1 or 2, with reference to the midpoint of the Physician's Desk Reference-recommended daily dose range for each medication. We generated a composite measure of medication load by summing all individual medication codes for each medication category for each individual participant. This strategy has been employed in our previous neuroimaging studies in BD (1-3).

Data acquisition

Neuroimaging data were collected using a 3.0 Tesla Siemens Allegra MRI scanner at the University of Pittsburgh/CMU Brain Imaging Research Center. Structural 3D sagittal MPRAGE

images were acquired in the same session (TE : 2.48 ms, TR: 1630 ms, Flip angle 8°, Field of view: 200 mm, Slice thickness: 1 mm, Matrix: 256 x 256, 192 continuous slices). BOLD functional images were then acquired with a gradient echo EPI sequence covering 33 axial slices (3 mm thick, 0 mm gap; TR/TE = 2000/25 msec, FOV = 24 cm, matrix = 64 x 64). All scanning parameters were selected to optimize the BOLD signal quality while maintaining a sufficient number of slices to acquire whole-brain data.

Exploratory whole brain neuroimaging data analyses

A second-level random-effects group analysis was conducted on the t-contrast images generated in the previous single-subject analyses in a 4 (group) by 3 (emotion intensity condition) repeated-measures analysis of variance (ANOVA) to explore significant whole brain neural activity in main effect of group and group by condition interaction. Clusters were considered significant if they survived inferences made at first $p<0.001$, uncorrected for multiple comparisons, and second, a minimum of three voxels within the cluster.

Within group analysis in the amygdala

Sad experiment

Within group analysis revealed significant left amygdala activity to intense sad faces and bilaterally in response to neutral faces in BD depressed individuals (BDd) (Table S1).

Fear experiment

Within group analysis revealed significant amygdala activation in the left side in response to mild fear faces in the BD depressed individuals, bilaterally in response to intense fear faces and

right sided in response to neutral faces in the BD remitted individuals (BDr), and bilaterally in response to neutral faces in the healthy control (HC) individuals (Table S1).

Happy experiment

Within group analysis revealed significant amygdala activation in the right side in response to intense happy faces in the BD depressed individuals, and left sided in response to intense and mild happy faces in the MDD depressed individuals (Table S1).

Females only

Since the majority of the participants were females, we also performed a region of interest (ROI) analysis with the female subgroup (Table S5). Similarly with our principal results, only a main effect of group to the left amygdala during the sad experiment was significant (coordinates: x=-21, y=-3 and z=-18; k=9 voxels; $F(3, 135)=3.59$; $p=0.015$). Post hoc analyses for mean amygdala activity to all emotion intensities indicated significantly greater left amygdala activity in BDd versus BDr ($t(22)=4.4$; $p<0.001$; $d=1.84$), BDd versus depressed recurrent MDD individuals ($t(25)=3.4$; $p=0.002$; $d=1.31$), and versus HC ($t(24)=3.5$; $p=0.002$; $d=1.35$), over all emotional intensities. Further analyses revealed that the significant increase in left amygdala activity in BDd versus BDr, versus depressed recurrent MDD individuals, and versus HC was evident to neutral expressions: BDd versus BDr: neutral: $t(22)=3.9$; $p=0.001$; $d=1.63$; BDd versus depressed recurrent MDD individuals: neutral: $t(25)=3.3$; $p=0.003$; $d=1.28$; BDd versus HC: neutral: $t(24)=3.5$; $p=0.002$; $d=1.38$.

Exploratory whole brain analyses

During the Happy experiment, whole brain ANOVA revealed a significant group by emotion interaction in: right hippocampus, right parahippocampal gyrus, right anterior cingulate, right temporal lobe and left posterior cingulate gyrus (Table S6).

During the Fear experiment, whole brain ANOVA revealed a significant main effect of group: right cuneus and left fusiform gyrus; and a group by emotion interaction: right inferior frontal gyrus and right thalamus (Table S6).

During the Sad experiment, whole brain ANOVA revealed a significant main effect of group: right cuneus and precuneus, left lingual gyrus and left inferior parietal lobule; and a group by emotion interaction: left amygdala and right parahippocampal gyrus (Table S6).

Table S1. Within group patterns of amygdala activity during the Happy, Sad and Fear experiments

			MNI Coordinates						
Region		**Side**	**X**	**Y**	**Z**	**k**	**t**	**z**	**p**
Happy Experiment									
BDd									
Amygdala	Intense	R	18	-6	-15	10	2.19	2.17	0.015
MDDd									
Amygdala	Intense	L	-21	-9	-9	9	2.22	2.20	0.014
	Mild	L	-21	-3	-18	15	2.27	2.25	0.012
Sad Experiment									
BDd									
Amygdala	Intense	L	-21	-3	-15	17	3.07	3.02	0.001
	Neutral	L	-15	-6	-15	25	4.27	4.15	<0.001
		R	24	0	-18	9	2.28	2.26	0.012
Fear Experiment									
BDd									
Amygdala	Mild	L	-18	-6	-18	18	2.55	2.53	0.006
BDr									
Amygdala	Intense	L	-21	-3	-18	9	3.19	3.13	0.001
		R	21	-3	-15	10	3.11	3.06	0.001
	Neutral	R	18	-6	-15	16	2.72	2.69	0.004
HC									
Amygdala	Neutral	L	-24	-3	-15	9	2.09	2.07	0.019
		R	18	-6	-21	9	2.43	2.41	0.008

R, right; L, left; HC, healthy control participants; MDDd, major depressive disorder patients in depressed episode; BDd, bipolar disorder patients in depressed episode; BDr, bipolar disorder patients in remission.

k: cluster size; Coordinates correspond to the stereotaxic array of Montreal Neurologic Institute.

Table S2. Relation between left amygdala activity, clinical, demographic and experiment performance variables in BD in the Fear experiment

	BDd (n=15) Left Amygdala Activity						**BDr (n=15) Left Amygdala Activity**					
	Fear Experiment						**Fear Experiment**					
	Intense		Mild		Neutral		Intense		Mild		Neutral	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
Age at Scan	**-0.66**	**0.008**	*-0.48*	*0.07*	**-0.52**	**0.05**	0.18	0.52	0.28	0.31	*0.46*	*0.08*
Age of Illness Onset	-0.33	0.23	-0.28	0.32	-0.23	0.41	-0.24	0.39	-0.17	0.54	0.19	0.49
Illness Duration	*-0.45*	*0.09*	-0.29	0.29	-0.38	0.16	**0.55**	**0.03**	**0.61**	**0.016**	0.43	0.11
HRSD-25	0.04	0.90	0.07	0.80	0.21	0.45	0.18	0.53	0.28	0.31	0.09	0.75
Medication Load	-0.13	0.64	-0.35	0.20	-0.38	0.16	-0.21	0.44	0.39	0.15	0.14	0.61
Face Expression Accuracy	-0.17	0.55	0.10	0.72	0.27	0.33	-0.42	0.12	-0.37	0.18	-0.37	0.17

	t	df	p	t	df	p	t	df	p	t	df	p	t	df	p	t	df	p
Gender (Male/Female)	-0.24	13	0.81	0.06	13	0.95	0.15	13	0.89	-0.54	13	0.6	-1.4	13	0.2	-0.02	13	0.9
Mood Stabilizers (ON/OFF)	-0.272	13	0.79	-1.1	13	0.29	-0.4	13	0.7	0.56	13	0.6	0.89	13	0.4	1.4	13	0.2
Antipsychotics (ON/OFF)	0.099	13	0.92	-1.2	13	0.27	-1.2	13	0.24	-0.2	13	0.8	1.57	13	0.1	0.48	13	0.6
Antidepressants (ON/OFF)	-0.641	13	0.53	-0.7	13	0.51	-0.9	13	0.37	-0.27	13	0.8	0.26	13	0.8	0.53	13	0.6
Benzodiazepines (ON/OFF)	-0.805	13	0.44	-1.6	13	0.13	-0.7	13	0.51	-0.15	13	0.9	0.3	13	0.8	0.62	13	0.5
Lifetime Presence of Alcohol/Drugs Abuse or Dependence (YES/NO)	0.1052	4	0.92	-0.1	11	0.93	0.66	11	0.52	0.74	13	0.5	0.47	13	0.6	-0.5	13	0.6

BDd, bipolar disorder patients in depressed episode; BDr, bipolar disorder patients in remission; HRSD-25, 25-item Hamilton Rating Scale for Depression.
r: Pearson correlation; bold: survive statistical threshold; italic: trend level.

Table S3. Relation between left amygdala activity, clinical, demographic and experiment performance variables in MDDd in the Fear experiment

	MDDd (n=15) Left Amygdala Activity					
	Fear Experiment					
	Intense		Mild		Neutral	
	r	p value	r	p value	r	p value
Age at Scan	0.18	0.53	0.13	0.65	-0.12	0.67
Age of Illness Onset	-0.08	0.77	-0.10	0.71	-0.11	0.71
Illness Duration	0.24	0.39	0.21	0.46	-0.04	0.89
HRSD-25	0.31	0.26	0.31	0.26	0.35	0.20
Medication Load	0.32	0.24	0.33	0.22	0.43	0.11
Face Expression Accuracy	0.04	0.87	0.05	0.86	-0.01	0.98

	t	df	p	t	df	p	t	df	p
Gender (Male/Female)	1.11	13	0.29	0.8	13	0.44	1.14	13	0.27
Antipsychotics (ON/OFF)	0.8731	13	0.4	0.64	13	0.53	1.49	13	0.16
Antidepressants (ON/OFF)	0.7811	13	0.45	1.4	13	0.19	0.7	13	0.5
Benzodiazepines (ON/OFF)	-0.21	13	0.84	-0.1	13	0.95	0.47	13	0.64
Lifetime Presence of Alcohol/Drugs Abuse or Dependence (YES/NO)	0.0566	13	0.96	-1	13	0.35	-0.1	13	0.89

MDDd, major depressive disorder patients in depressed episode; HRSD-25: 25-item Hamilton Rating Scale for Depression.

r: Pearson correlation.

Table S4. Relation between left amygdala activity, demographic and experiment performance variables in HC in the Sad and Fear Experiment

	HC (n=15) Left Amygdala Activity											
	Sad Experiment											
	Mean			Intense			Mild			Neutral		
	r	p value		r	p value		r	p value		r	p value	
Age at Scan	0.11	0.70		0.12	0.66		0.13	0.64		0.02	0.95	
Accuracy Emotional Face	0.35	0.20		0.26	0.35		*0.47*	*0.08*		-0.02	0.94	
Accuracy Neutral Face	0.14	0.61										
				t	df	p	t	df	p	t	df	p
Gender (Male/Female)	*1.96*	*13*	*0.07*	0.37	13	0.7	1.03	13	0.3	2.09	2	0.16

	Fear Experiment								
	Intense			Mild			Neutral		
	r	p value		r	p value		r	p value	
Age at Scan	-0.36	0.18		-0.48	0.07		-0.15	0.60	
Accuracy Emotional Face	-0.41	0.13		-0.35	0.20		-0.21	0.44	
Accuracy Neutral Face									
	t	df	p	t	df	p	t	df	p
Gender (Male/Female)	-1	13	0.3	-0.2	13	0.9	-1.5	13	0.2

HC, healthy control.
italic: trend level; r: Pearson correlation.

Table S5. Post hoc between-group comparison in amygdala activity in the Sad experiment in the female subgroup, and in the significant left amygdala cluster arising in the group x emotion interaction in whole brain analyses

	BDd > BDr			**BDd > MDDd**			**BDd > HC**			**BDr > MDDd**			**BDr > HC**			**MDDd > HC**		
Sad Experiment - Females only																		
	t^c	p	d	t^d	p	d	t^e	p	d	t^f	p	d	t^g	p	d	t^h	p	d
Mean Left Amygdala[a]	**4.4**	**<0.001**	**1.84**	**3.4**	**0.002**	**1.31**	**3.5**	**0.002**	**1.35**	-0.8	0.4	0.33	-0.8	0.4	0.35	-0.02	1.0	<0.01
Intense Left Amygdala[b]	1.5	0.2	0.61	1.6	0.1	0.62	*2.0*	*0.05*	*0.80*	0.1	0.9	0.05	0.6	0.5	0.27	0.5	0.6	0.2
Mild Left Amygdala[b]	*2.6*	*0.02*	*1.03*	*2.5*	*0.02*	*0.96*	*2.0*	*0.05*	*0.79*	-0.4	0.7	0.18	-1.0	0.3	0.43	-0.7	0.5	0.3
Neutral Left Amygdala[b]	**3.9**	**0.001**	**1.63**	*3.3*	*0.003*	*1.28*	**3.5**	**0.002**	**1.38**	-1.4	0.2	0.56	-1.3	0.2	0.56	0.1	0.9	<0.01
Sad Experiment – Whole brain interaction																		
	t^i	p	d	t^i	p	d	t^i	p	d	t^i	p	d	t^i	p	d	t^i	p	d
Mean Left Amygdala[a]	**5.9**	**<0.001**	**2.14**	*2.4*[g]	*0.02*	*0.89*	1.9[g]	0.07	0.68	*-2.0*	*0.05*	*0.73*	*-2.3*	*0.03*	*0.85*	-0.4	0.7	0.1
Intense Left Amygdala[b]	-0.2	0.9	0.05	-0.2	0.9	0.07	0.9	0.4	0.34	-0.1	0.9	0.03	1.1	0.3	0.41	0.9	0.4	0.3
Mild Left Amygdala[b]	**4.8**	**<0.001**	**1.77**	*3.1*	*0.004*	*1.14*	0.9[g]	0.4	0.34	*-2.2*	*0.03*	*0.81*	*-2.9*	*0.01*	*1.07*	-1.2	0.2	0.5
Neutral Left Amygdala[b]	**6.1**	**<0.001**	**2.23**	*2.9*	*0.008*	*1.05*	*2.4*	*0.03*	*0.86*	*-2.1*	*0.05*	*0.76*	*-2.6*	*0.02*	*0.95*	-0.4	0.7	0.2

BDd, bipolar disorder patients in depressed episode; BDr, bipolar disorder patients in remission; MDDd, major depressive disorder patients in depressed episode; HC, healthy controls

bold: survive statistical threshold; italic: trend level

[a] statistical threshold at p<0.0083

[b] statistical threshold at p<0.0028

[c] degree of freedom equal to 22 unless otherwise specified

[d] degree of freedom equal to 25

[e] degree of freedom equal to 24

[f] degree of freedom equal to 21

[g] degree of freedom equal to 20

[h] degree of freedom equal to 23

[i] degree of freedom equal to 28

Table S6. Exploratory whole brain activity during the Happy, Sad and Fear experiments

		MNI Coordinates						
Region	**Side**	**X**	**Y**	**Z**	**k**	**F**	**z**	**p**
Happy Experiment								
Interaction								
Hippocampus	R	33	-36	3	4	5.53	4.01	<0.001
Parahippocampal Gyrus (BA30)	R	27	-51	6	3	4.92	3.68	<0.001
Anterior Cingulate (BA24)	R	3	33	9	4	4.70	3.56	<0.001
Temporal Lobe (BA41)	R	36	-33	12	3	4.57	3.48	<0.001
Posterior Cingulate Gyrus (BA31)	L	-18	-33	39	3	4.19	3.24	0.001
Sad Experiment								
Main Effect of Group								
Cuneus (BA18)	R	12	-90	18	12	8.84	4.13	<0.001
Precuneus (BA19)	R	36	-72	45	6	7.16	3.62	<0.001
Lingual Gyrus (BA18)	L	-9	-84	6	5	6.55	3.41	<0.001
Inferior Parietal Lobule (BA40)	L	-39	-54	45	3	6.02	3.22	0.001
Interaction								
Amygdala	L	-12	-3	-15	9	5.84	4.17	<0.001
Parahippocampal Gyrus (BA30)	R	6	-42	-6	3	4.77	3.59	<0.001
Fear Experiment								
Main Effect of Group								
Cuneus (BA18)	R	12	-90	21	3	7.04	3.58	<0.001
Fusiform Gyrus (BA37)	L	-27	-51	-12	4	6.01	3.22	<0.001
Interaction								
Inferior Frontal Gyrus (BA47)	R	39	33	-18	3	5.76	4.13	<0.001
Thalamus	R	21	-3	9	4	4.61	3.50	<0.001

R, right; L, left; BA, Brodmann area.
k: cluster size; Coordinates correspond to the stereotaxic array of Montreal Neurologic Institute.

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