



Effects of arterial cannulation stress on regional cerebral blood flow in major depressive disorder

SUBJECT AREAS:
NEUROANATOMY
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Jonathan Savitz^{1,2,3}, Allison C. Nugent^{1,4}, Dara M. Cannon⁵, Paul J. Carlson⁶, Rebecca Davis¹, Alexander Neumeister⁷, Denise Rallis-Frutos¹, Steve Fromm¹, Peter Herscovitch⁸ & Wayne C. Drevets^{1,2,9}

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Correspondence and requests for materials should be addressed to J.S. (jonathansavitz@hotmail.com)

¹Section on Neuroimaging in Mood and Anxiety Disorders, Mood and Anxiety Disorders Program, NIH/NIMH, Bethesda, MD, ²Laureate Institute for Brain Research, Tulsa, OK, ³Department of Medicine, Tulsa School of Community Medicine, Tulsa, OK, ⁴Experimental Therapeutics, Mood and Anxiety Disorders Program, NIH/NIMH, Bethesda, MD, ⁵Clinical Neuroimaging Laboratory, Psychiatry, National University of Ireland Galway, Ireland, ⁶Salt Lake City VA and Department of Psychiatry, University of Utah, Salt Lake City, UT, ⁷Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, ⁸Clinical Center, National Institutes of Health, Bethesda, MD, ⁹The University of Oklahoma College of Medicine, Department of Psychiatry, Tulsa, OK.

Individuals with major depressive disorder (MDD) display abnormal neurophysiological responses to psychological stress but little is known about their neurophysiological responses to physiological stressors. Using [¹⁵O]-H₂O positron emission tomography we assessed whether the regional cerebral blood flow (rCBF) response to arterial cannulation differed between patients with MDD and healthy controls (HCs). Fifty-one MDD patients and 62 HCs were scanned following arterial cannulation and 15 MDD patients and 17 HCs were scanned without arterial cannulation. A region-of-interest analysis showed that a significantly increased rCBF of the anterior cingulate cortex and right amygdala was associated with arterial cannulation in MDD. A whole brain analysis showed increased rCBF of the right post-central gyrus, left temporopolar cortex, and right amygdala during arterial cannulation in MDD patients. The rCBF in the right amygdala was significantly correlated with depression severity. Conceivably, the limbic response to invasive physical stress is greater in MDD subjects than in HCs.

Individuals with major depressive disorder (MDD) show altered behavioral¹, neuroendocrine², autonomic³, and neurophysiological⁴ responses to psychological stress. Neuroimaging paradigms have therefore been developed which implicitly or explicitly attempt to mimic aspects of the social environment in order to better model neural responses to psychosocial stress, and thereby improve our understanding of MDD. Stimuli have taken the form of emotionally-valenced words or visual stimuli, sad, angry or frightened faces, and recordings of critical comments.

In aggregate, the results of these studies suggest that MDD is characterized by a dysregulation of the visceromotor network, which regulates endocrine and behavioral responses to stress⁵. In particular, there is evidence for a reduction in “top-down” cortical (prefrontal cortex) control over structures involved in mediating the visceral and behavioral aspects of emotional behavior, such as the anterior insula (AI), ventral striatum, mediodorsal thalamus, hippocampal subiculum, amygdala, hypothalamus and brain-stem nuclei.

Nevertheless, the functional imaging data are largely based on responses to what are, by practical and ethical necessity, relatively subtle and situationally-constrained emotional or psychosocial interventions rather than responses to both non-social and physiological stressors outside of the laboratory. One such environmental stressor, which has been understudied in patients who meet DSM-IV criteria for MDD, is the response to robust physiological stressors involving pain or mildly invasive procedures.

While conducting PET neuroreceptor mapping studies of MDD, our laboratory routinely acquired¹⁵O-H₂O regional cerebral blood flow (rCBF) scans prior to neuroreceptor imaging to facilitate data processing and/or tracer modeling. For some of these studies, radial arterial cannulation is necessitated by the need for an arterial input function. This mildly invasive procedure typically induces mild-to-moderate levels of pain (despite the application of local anesthetic agents), possibly via stimulation of the radial nerve⁶, and activates the neural systems implicated in stress biology, including the limbic-hypothalamic-pituitary-adrenal (HPA) axis⁷, resulting in elevated cortisol⁸, and a vascular sympathetic autonomic response. Since a tight coupling (positive correlation) exists between changes in rCBF and local terminal field metabolic activity^{9,10}, the methodological requirements of



these neuroreceptor PET studies afforded us an opportunity to study the neurophysiological response of MDD patients and healthy controls to a more invasive and aversive stressor than has been used in previous neuroimaging studies.

The aim of our current study was to test whether neurophysiological regulation of response to an unpleasant physiological stressor is modified by MDD. Specifically, we aimed to test whether compared to HC subjects, MDD patients show a greater difference in rCBF between high and low stress experimental conditions. That is, are depressed subjects more sensitive neurophysiologically to an invasive stressor than healthy controls?

Stress responses are mediated by the sympathetic-adrenomedullary (SAM) and the HPA axes, resulting in increased release of epinephrine, norepinephrine, and cortisol, respectively¹¹. In two independent cohorts we previously found that compared to healthy controls, depressed patients show increased cortisol release in response to arterial cannulation^{8,12}. Limbic structures such as the amygdala, which project directly to the paraventricular nucleus (PVN) of the hypothalamus, regulate stress-induced glucocorticoid secretion by disinhibiting cortisol-releasing factor (CRF) release from the PVN¹³. Conversely, there is an extensive body of research demonstrating that epinephrine, norepinephrine, and cortisol influence amygdala function^{14,15}. Based on these data, as well as the extensive literature on the role of the amygdala in depression and pain, we expected to find increased rCBF of the amygdala in MDDart+ patients.

This study also specifically investigates two regions of interest (ROIs) comprising the cortical components of the stress matrix that process the emotional, endocrine and autonomic dimensions of aversive stimulation - the anterior cingulate (ACC) and anterior insular cortices^{16,17}. In their meta-analysis, Apkarian et al. show that 45 out of 68 studies of pain induction in healthy subjects reported activity changes in insula, the primary viscerosensory and nociceptive cortex¹⁸. This meta-analysis also noted changes in ACC activity in 46 of 68 studies, consistent with evidence that the ACC modulates amygdala-driven CRF release¹⁹ in response to noxious emotional or physiological stimuli¹⁸.

Results

Demographic and Clinical Variables. These data are shown in Table 1.

There was no significant difference between the 4 groups in gender distribution ($\chi^2=1.0$, $p=0.81$) or age at scan ($F=1.9$, $p=0.13$). No significant difference was found between the MDD patients who underwent arterial cannulation (MDDart+) and MDD patients who did not undergo arterial cannulation (MDDart-) groups on the number of weeks medication free ($t=1.2$, $p=0.24$), age at onset ($t=0.7$, $p=0.50$), or illness duration ($t=1.2$, $p=0.25$).

Both the art+ and the art- MDD groups scored higher than the art+ and art- HC groups on the MADRS ($F=215$, $p<0.001$) but there was no significant difference between the art+ and art- MDD

Table 1 | Demographic and Clinical Characteristics of the Sample

Trait	Diagnosis				Statistics			
	MDD_art	MDD_no_art	HC_art	HC_no_art	Statistic	DF	p-value	Result
N	51	15	62	17				
Gender					$\chi^2=1.0$	3	0.81	NS
Males	19	6	28	6				
Females	32	9	34	11				
Percent Female	63	60	55	65				
Age at Scan	37.6±9.7	31.9±10.7		34.8±9.6	31.9±8.4	F=1.9	3	0.13
NS								
Past Medication Use (>3 weeks previous; N)			NA	NA				
Naïve	20	10						
AD	27	5						
Mood Stabilizers	7	2						
Benzodiazepines	2	1						
Weeks Medication Free	93.4±120.7	27.7±9.3	NA	NA	t=1.2	32	0.24	NS
Age at Onset	18.0±6.2	19.8±11.7	NA	NA	t=0.7	48	0.50	NS
Years ill	17.8±11.4	13.7±9.7	NA	NA	t=1.2	48	0.25	NS
Family History of Affective Illness (N)								
Present	31	11	0	0				
Absent	4	1	62	17				
Uncertain	16	3	0	0				
Past History of Substance/Alcohol Abuse (N)			NA	NA				
Present	8	2						
Absent	43	13						
Common Comorbid Disorders in Sample (N)			NA	NA				
Panic Disorder	5	3						
PTSD	4	4						
GAD/Social Anxiety Disorder	3	2						
Eating Disorder	2	1						
Mood Ratings								
MADRS	24.7±8.1	22.5±5.4	0.3±0.7	0.5±1.2	t=1.0 ^a and t=0.7 ^b	53 ^a and 68 ^b	0.34 ^a and 0.49 ^b	NS

Note: (a) comparison of MDDart+ and MDDart- on the MADRS.
(b) comparison of HCart+ and HCart- on the MADRS.



groups ($t=1.0$, $p=0.34$) or between the art+ and art- HC groups ($t=0.7$, $p=0.49$) on the MADRS.

Although no demographic or clinical variables differed significantly across the 4 subject groups, the MDD art+ subjects were on average 5.5 years older than the MDD art- subjects. Similarly, the HC art+ group was on average 3 years older than the HC art- group. To ensure that our results were not systematically biased by age effects, we therefore added age as a covariate in the general linear model.

Imaging. ROI Analysis. *Contrast A: MDDart+ versus MDDart-*. MDDart+ patients displayed *higher* rCBF compared with MDDart- patients in the following regions (table 2 and fig. 1A): the supragenual and pregenual ACC, left posterior insula, and left and right amygdalae.

MDDart+ patients displayed *lower* rCBF compared with MDDart- patients in the following regions (table 2 and fig. 1B): left and right anterior insulae and the sgACC.

Contrast B: HCart+ versus HCart-. Within the predefined ROI no areas were identified where rCBF differed significantly between the HCart+ and HCart- groups.

Contrast C = A-B. The MDD patients showed a significant *increase* in rCBF in response to arterial cannulation in the right amygdala and the supragenual ACC relative to healthy controls (table 3, fig. 1C). No significant *decrease* in the rCBF of MDD patients in response to arterial cannulation stress was observed relative to healthy controls.

In *post hoc* assessments the flow obtained over the locus where the peak voxel-t-value was evident within the right amygdala (table 3) was significantly correlated (positively) with the MADRS score in the

Table 2 | Results of the Region-of-Interest (ROI) analysis for contrast A showing areas of increased (top panel) or decreased (lower panel) rCBF in MDDart+ subjects versus MDDart- subjects

CONTRAST A - ROI Analysis										
Increased rCBF in MDDart+ vs. MDDart-										
Cluster Size	T score	Z score	p-value	FDR p-value	Coordinates ^a			Region of Interest ^b	Additional Comments	
					X	Y	Z			
2508	4.41	4.26	<0.001	0.006	-2	26	24	ACC, supragenual	Cluster size corrected p-value <0.001; FWE corrected p-value=0.018 Cluster size corrected p-value =0.002; FWE corrected p-value=0.031	
	4.25	4.12	<0.001	0.006	2	32	15	ACC, pregenual		
120	4.00	3.88	<0.001	0.006	2	15	25	ACC, supragenual		
	3.85	3.75	<0.001	0.006	24	1	-20	R amygdala		
232	3.83	3.73	<0.001	0.006	-40	-12	-6	Insula, L posterior insula		
	3.52	3.44	<0.001	0.007	-44	-19	5	Insula, L posterior insula		
72	3.39	3.32	<0.001	0.008	-24	1	-20	L amygdala		
159	3.39	3.32	<0.001	0.008	40	-17	-14	Insula, R posterior insula		
	3.33	3.26	0.001	0.009	42	-1	-12	"Insula", R temporopolar cortex		
	3.10	3.04	0.001	0.013	46	9	-11	"Insula" R temporopolar cortex		
CONTRAST A - ROI Analysis										
Decreased rCBF in MDDart+ vs. MDDart-										
Cluster Size	T score	Z score	p-value	FDR p-value	Coordinates ^a			Region of Interest ^b	Additional Comments	
					X	Y	Z			
385	4.51	4.35	<0.001	0.014	36	-1	15	"Insula", R dorsal insula/ frontal operculum	FWE corrected p-value = 0.014, Cluster corrected p-value = 0.036	
	3.53	3.45	<0.001	0.036	32	22	10	"Insula", R dorsal insula/ intrasulcal lateral OFC (BA47s)		
320	3.41	3.33	<0.001	0.028	-40	-7	17	"Insula", L dorsal insula/ frontal operculum	Cluster corrected p-value = 0.053	
	3.17	3.11	0.001	0.043	-40	18	6	"Insula", L dorsal insula/ frontal operculum		
34	3.04	2.99	0.001	0.050	-30	8	11	"Insula", L Anterior Insula		
	3.06	3.01	0.001	0.048	12	25	-10	Right ACC, subgenual		

a. Coordinates correspond to the stereotaxic array of Talairach and Tournoux⁵⁵ and are in mm from the stereotaxic origin (anterior commissure) with positive x = right, positive y = anterior, and positive z = dorsal to a plane containing both the anterior and posterior commissures.

b. The region-of-interest in which differences in normalized flow is listed first; however, because the regional template for the insula extended outside the cortex of primary interest to encompass the entire operculum, where the coordinates localized cortical areas other than the insula based on the stereotaxic coordinates (Mai et al.⁵⁴; Talairach and Tournoux⁵⁵; Ongur et al.²⁹) these cortices are named accordingly and the ROI name appears in quotation marks (i.e., "insula").

Significance threshold for peak voxel in cluster: $p \leq 0.001$.

Significance threshold for contiguous voxels: $p \leq 0.01$ and region ≥ 100 mm³ in size.

Abbreviations: ACC= anterior cingulate cortex; L=left; OFC=orbitofrontal cortex; R=right; FDR=false discovery rate corrected p-value; FWE=family-wise error corrected p-value.

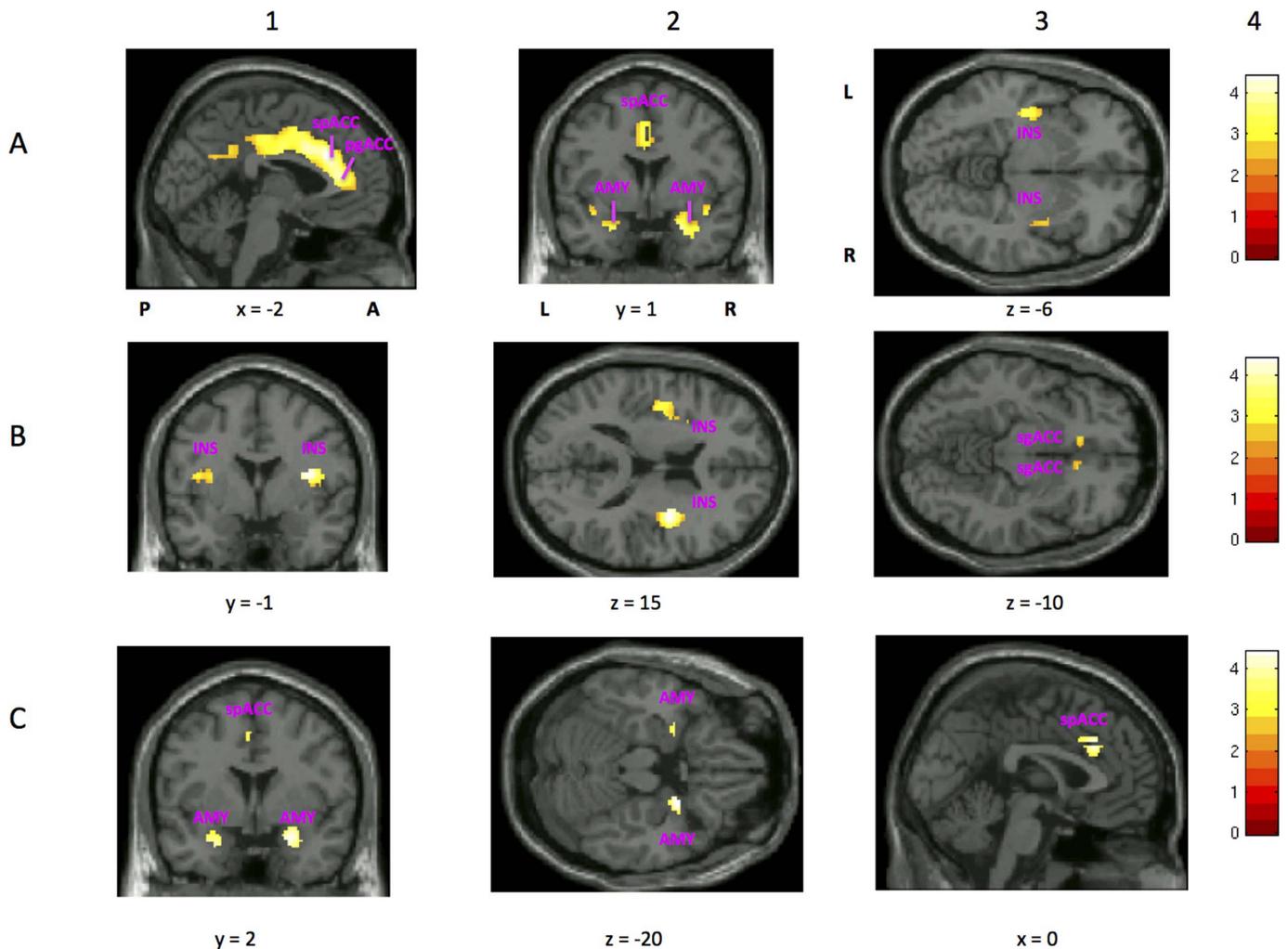


Figure 1 | Image sections from a statistical parametric map of voxel t-values which show regions where rCBF is significantly increased or decreased in contrasts A and C of the ROI analysis. Stereotaxic coordinates provided beneath each section image denote the distance in mm from the stereotaxic origin (anterior commissure), with positive x indicating right of midline (for sagittal sections), positive y indicating anterior (for coronal sections), and positive z indicating dorsal (for horizontal sections). Abbreviations: A=anterior to anterior commissure, P=posterior to anterior commissure, L=left hemisphere, R=right hemisphere, spACC=supragenual ACC, pgACC=pregenual ACC, AMY=amygdala, INS=insula, sgACC=subgenual ACC. Row A shows regions of the brain that show greater rCBF in MDDart+ versus MDDart- patients (contrast A). A1: sagittal slice showing areas where voxel t-values show significantly increased activity in the supragenual and pregenual ACC ($x = -2$, $y = 26$, $z = 24$). The posterior ACC is also evident but is below the significance threshold; A2: coronal slice showing higher activity in the amygdala, bilaterally, and in the supragenual ACC ($x = 24$, $y = 1$, $z = -20$); A3: axial slice showing elevated activity of the posterior insula, bilaterally ($x = -40$, $y = -12$, $z = -6$). Row B shows regions of the brain that demonstrate reduced flow in the MDDart+ group relative to the MDDart- group (contrast A). B1 and B2 display: coronal and axial slices, respectively, showing areas of decreased rCBF in the dorsal anterior insula/frontal operculum, bilaterally ($x = 36$, $y = -1$, $z = 15$), while B3 displays an axial slice showing decreased rCBF in the sgACC ($x = 12$, $y = 25$, $z = -10$) MDDart+ group relative to the MDDart- group. Row C shows regions of the brain associated with greater rCBF in response to arterial cannulation in the MDDart+ group compared with the MDDart- group relative to differences between the HCart+ and HCart- groups (contrast C). C1: coronal slice showing areas where flow is increased in the amygdala, bilaterally ($x = 24$, $y = 2$, $z = -20$), C2: axial slice showing areas of increased flow of the amygdala, bilaterally ($x = 24$, $y = 2$, $z = -20$), C3: sagittal slice showing region of peak rCBF in the supragenual ACC ($x = 0$, $y = 27$, $z = 28$). A4, B4, and C4: Key showing correspondence between image color and voxel t-value.

MDDart+ group ($r = 0.272$, $p = 0.045$). In the left amygdala the normalized activity showed a nonsignificant trend towards correlating positively with the MADRS score ($r = 0.231$, $p = 0.076$) (fig. 3). There was no significant association between the MADRS scores and normalized activity of the right amygdala ($r = 0.036$, $p = 0.449$) and left amygdala ($r = -0.33$, $p = 0.454$) in the MDDart- group. There was also no significant correlation between the normalized activity of the peak voxel in the supragenual ACC cluster and MADRS score in either the MDDart+ ($r = 0.002$, $p = 0.988$) or the MDDart- ($r = -0.254$, $p = 0.361$) groups.

Contrast D: MDDart- versus HCart-. Within the predefined ROI no areas were identified where the MDDart- group showed greater rCBF

than the HCart- group. The MDDart- group did however show reduced rCBF in the left insula compared with the HCart- group (table 4).

Whole Brain Analysis. Contrast A: MDDart+ versus MDDart-. Regions in which MDDart+ patients showed *increased* rCBF compared with MDDart- participants included (table 5 and fig. 2A): the supragenual and pregenual ACC, right entorhinal cortex, temporopolar cortex bilaterally, rostral temporal sulcal cortex (junction of superior and middle temporal gyri) bilaterally, ventromedial PFC, left ventrolateral PFC, left lateral orbitofrontal PFC, postcentral gyri and adjacent inferior parietal cortex bilaterally, and precuneus.



Table 3 | Region-of-interest analysis showing *increased* rCBF in the MDD sample versus the HC sample in the high versus low stress condition (contrast C)^a

CONTRAST C – ROI Analysis									
Cluster Size	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region of Interest	Additional Comments
					X	Y	Z		
88	3.30	3.23	0.001	0.330	24	2	-20	R amygdala	L amygdala significant at peak voxel threshold of $p \leq 0.002$ (cluster size 51)
140	3.17	3.11	0.001	0.330	0	27	28	ACC, supragenual ACC	

Significance threshold for peak voxel in cluster: $p \leq 0.001$.
 Significance threshold for contiguous voxels: $p \leq 0.01$ and region $\geq 100 \text{ mm}^3$ in size.
 Abbreviations and coordinates interpreted as in table 2.
 a. No regions showed statistically significant *decreases* in rCBF in the MDD sample versus the HC sample.

MDD art+ patients showed *reduced* rCBF compared with the MDD art- patients in the following regions (table 5 and fig. 2B): right anterior insula, left insula, and left frontal operculum.

Contrast B: HCart+ versus HCart-. Relative to the HCart- group the HCart+ group showed higher rCBF in the right premotor cortex and lower rCBF in the left parahippocampal gyrus (table 6).

Contrast C = A–B. The MDD patients showed a significant *increase* in rCBF compared with HC subjects in response to arterial cannulation in the following regions (table 7, fig. 2C): the right postcentral gyrus, right amygdala, and left temporopolar cortex.

No regions of the brain showed a *decrease* in rCBF in MDD patients compared with HC subjects in response to the arterial line stressor.

In *post hoc* assessments there was no significant correlation between the normalized activity at the peak voxel in the left temporopolar cortex and the MADRS scores in either the MDDart+ ($r=0.108$, $p=0.508$) or the MDDart- ($r=-0.084$, $p=0.766$) groups. In addition, there was no significant correlation between the normalized activity at the peak voxel in the right post-central gyrus and the MADRS scores in either the MDDart+ ($r=0.154$, $p=0.344$) or the MDDart- ($r=-0.208$, $p=0.457$) groups. The corresponding relationship at the peak voxel in the right amygdala was not tested because of its proximity to the peak coordinate in the right amygdala in the ROI analysis (see below).

Contrast D: MDDart- versus HCart-. The MDDart- group showed a significant *increase* in rCBF compared with HCart- subjects in the right posterior hippocampus (table 8).

No regions of the brain showed a significant *decrease* in rCBF in MDDart- patients compared with HCart- subjects.

Discussion

The main focus of this study was to determine if there is a pattern of rCBF that distinguishes MDD patients from healthy individuals

when they are subjected to an invasive stressor. In general, depressed subjects showed neurophysiological responses to arterial cannulation stress that were exaggerated relative to those of healthy controls in limbic/paralimbic regions of the anterior cingulate cortex, amygdalae and temporopolar cortex. Our most salient results were as follows:

- (1) In the supragenual, pregenual ACC, and the ventromedial prefrontal cortex, rCBF increased in MDD patients in the arterial cannulation versus the control condition (contrast A: table 2, fig. 1A; table 4, fig. 2A), while the corresponding contrast in healthy controls (contrast B) revealed no significant difference in these areas. These regions form part of a medial prefrontal or “visceromotor” network that plays major roles in modulating the visceral (autonomic, endocrine) responses and processing associated with stress and emotion²⁰. Additionally, after controlling for differences in rCBF in healthy subjects across high and low stress conditions, a more circumscribed region of the ACC showed greater rCBF in MDD patients compared with HC subjects (contrast C: table 3, fig. 1C). We have labeled this region as the “supragenual ACC” to signify that it is immediately superior to the genu of the corpus callosum but a variety of different terms – ACC, rostral ACC, anterior midcingulate cortex, perigenual ACC, and pregenual ACC– have been used to describe this region in the literature. The supragenual/pregenual ACC has been hypothesized to be the human homologue of the rat prelimbic cortex which exerts a predominantly inhibitory effect on HPA function via its connections with the bed nucleus of the stria terminalis and the paraventricular nucleus of the hypothalamus¹⁹, and is highly sensitive to psychological²¹ and physiological stress²². In humans, this region is also activated by noxious stimuli, particularly visceral as opposed to cutaneous stimuli, as well as psychological stressors, particularly those associated with the emotion of fear²³. Critchley et al²⁴ showed that neurophysiological activity in the supragenual and pregenual ACC

Table 4 | Region-of-interest analysis for contrast D showing increased rCBF in the MDD sample versus the HC sample in the low stress condition (i.e. neither group underwent arterial cannulation)^a

CONTRAST D – ROI Analysis									
Cluster Size	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region of Interest	
					X	Y	Z		
33	3.35	3.27	0.001	0.420	-43	-26	4	“Insula”, L temporopolar cortex	

Significance threshold for peak voxel in cluster: $p \leq 0.001$.
 Significance threshold for contiguous voxels: $p \leq 0.01$ and region $\geq 100 \text{ mm}^3$ in size.
 Coordinates are interpreted as in table 2.
 a. No regions showed statistically significant *decreases* in rCBF in the MDD sample versus the HC sample.



Table 5 | Results of the whole brain analysis for contrast A showing regions of increased (top panel) and decreased (bottom panel) rCBF in MDDart+ subjects versus MDDart- subjects

CONTRAST A – Whole Brain Analysis									
Increased rCBF in MDDart+ vs. MDDart-									
Cluster Size ^a	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region	
					X	Y	Z		
1175	4.41	4.26	<0.001	0.040	-2	26	24	Supragenual ACC	
	4.25	4.12	<0.001	0.040	2	32	15	Pregeneral ACC	
	4.00	3.88	<0.001	0.040	-2	13	31	Supragenual ACC	
679	4.38	4.24	<0.001	0.040	22	7	-21	R entorhinal C	
	4.03	3.92	<0.001	0.040	42	14	-23	R temporopolar C	
	3.79	3.69	<0.001	0.040	44	1	-17	R rostral STG/MTG	
551	4.28	4.14	<0.001	0.040	-34	5	-22	L temporopolar C	
	3.93	3.82	<0.001	0.040	-40	-5	-13	L rostral STG/MTG	
	3.73	3.69	<0.001	0.040	-34	12	-34	L temporopolar C	
61	4.10	3.98	<0.001	0.040	-57	-33	33	L inferior parietal lobule	
72	3.84	3.72	<0.001	0.040	59	-25	34	R postcentral gyrus	
40	3.74	3.65	<0.001	0.040	50	-13	-33	R inferior temporal gyrus	
65	3.73	3.64	<0.001	0.040	0	26	-25	Ventromedial prefrontal C	
	3.41	3.33	<0.001	0.047	-2	40	-26	Ventromedial prefrontal C	
22	3.62	3.54	<0.001	0.042	-46	22	19	L ventrolateral prefrontal C	
36	3.57	3.51	<0.001	0.045	-42	-12	39	L precentral gyrus	
28	3.52	3.44	<0.001	0.045	-42	41	7	L ventrolateral prefrontal C	
	3.22	3.16	0.001	0.052	-40	45	-2	L lateral orbitofrontal C	
58	3.40	3.33	<0.001	0.047	2	-60	34	Precuneus	
31	3.47	3.40	<0.001	0.046	-20	38	26	L dorsolateral prefrontal C	

CONTRAST A – Whole Brain Analysis									
Decreased rCBF in MDDart+ vs. MDDart-									
Cluster Size ^a	T score	Z score	p-value	FDR p-value	Coordinates			Region	
					X	Y	Z		
171	4.51	4.35	<0.001	0.177	36	-1	15	"Insula", R dorsal insula/frontal operculum	
49	4.01	3.90	<0.001	0.195	26	20	6	"Insula", R dorsal insula/intrasulcal lateral OFC (BA47s)	
22	3.41	3.33	<0.001	0.403	-40	-7	17	"Insula", L dorsal insula/frontal operculum	

a. Note that the difference in cluster sizes shown here vis a vis those in table 2 reflects the constraint of the anatomical extent of the cluster by the ROI boundaries. Only the local maxima with the three highest voxel t-values within a given cluster are listed here, so these loci should be considered additive to those listed in Tables 2 and 3 in the manuscript. Significance threshold for peak voxel in cluster: $p \leq 0.001$, and cluster size of contiguous voxels for which $p \leq 0.001$ corresponding to $> 100 \text{ mm}^3$. Coordinates are interpreted as in table 2. Abbreviations: ACC – anterior cingulate cortex; C – cortex; STG/MTG – cortex along the superior temporal sulcus at the junction of superior temporal gyrus and middle temporal gyrus; PFC – prefrontal cortex.

correlated with heart rate variability across a variety of mildly stressful cognitive-behavioral tasks. The supragenual ACC is activated by pictures of spiders in phobic individuals²⁵, self-administered but not externally-administered noxious stimulation²⁶, the experience of vicarious pain²⁷, painful emotions such as social exclusion²⁸, and placebo analgesia²⁹, suggesting that it is involved in the cognitive and emotional modulation of threatening bodily stimuli. Consistent with this hypothesis, emotions such as anxiety and sadness intensify the negative hedonic quality of experimentally-induced pain, a phenomenon that is correlated with hemodynamic activity of the supragenual ACC³⁰. Further, approximately 75% of severely ill MDD patients treated with stereotactic lesions of the supragenual ACC region (dorsal anterior cingulotomy) obtain some benefit from the procedure³¹.

There are data to suggest that the stress-associated increase in supragenual and pregeneral activity may be related to HPA axis function. Hubbard et al.³² recently demonstrated that anxious patients with irritable bowel syndrome (IBS) show a strong positive coupling between BOLD response in the supragenual ACC and the amygdala during expectation of pain. Notably, administration of a CRF antagonist eliminated this positive coupling possibly by normalizing negative feedback by the amygdala on the pregeneral ACC³².

(2) Bilateral increases in rCBF of the amygdala were observed in the MDDart+ group compared with the MDDart- group (but not in the HCart+ versus the HCart- groups) in the ROI analysis (contrast A: table 2, fig. 1A). In contrast C, increased rCBF was observed in the right amygdala in the MDDart+ group compared with the MDDart- group, relative to the HCart+ group compared with the HCart- group. An increase in CBF in the left amygdala in the MDDart+ group compared with the MDDart- group relative to the corresponding difference in healthy controls also was observed at a peak voxel threshold of $p < 0.005$ (contrast C: table 3, fig. 1C). Further, an increase in CBF of the right amygdala in the MDDart+ versus MDDart- group relative to controls was observed in the whole brain analysis with a trend towards significance for the left amygdala (contrast C: table 4, fig. 2C). On average, MDDart+ patients who had higher MADRS scores showed significantly greater activity at the peak voxel in the right amygdala derived from contrasts A and C, than MDDart+ patients with lower MADRS scores. Similarly, there was a trend towards a significant positive correlation between activity at the peak voxel in the left amygdala and MADRS scores in the MDDart+ group (fig. 3). The whole brain analysis additionally showed that rCBF was higher in MDDart+ patients versus MDDart- patients (but not in

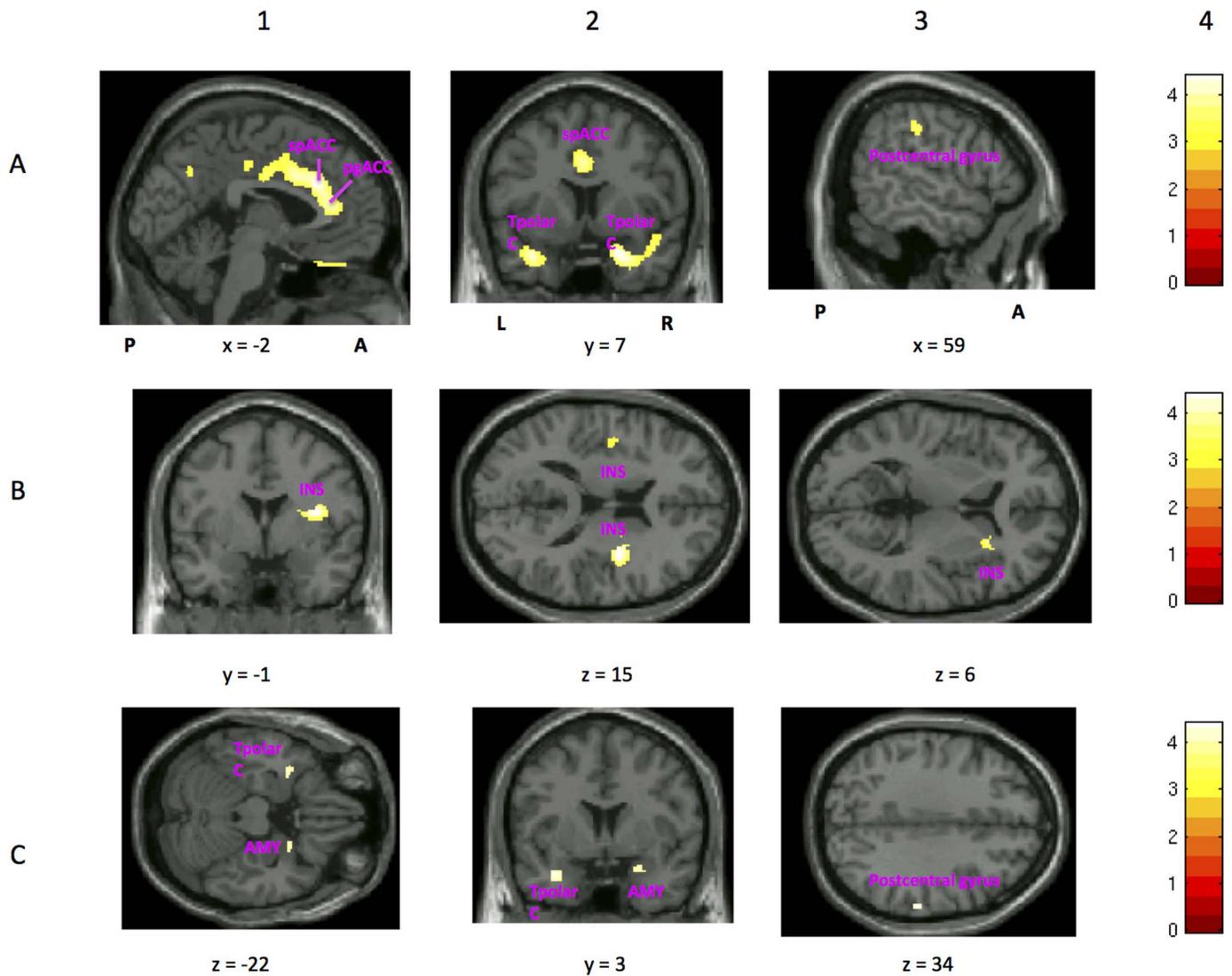


Figure 2 | Image sections from a statistical parametric map of voxel t-values which show regions where rCBF is significantly increased or decreased in contrasts A and C of the whole brain analysis. Stereotaxic coordinates are provided beneath each section, and are interpreted as described in the legend for figure 1. Abbreviations: A=anterior to anterior commissure, P=posterior to anterior commissure, L=left hemisphere, R=right hemisphere, spACC=supragenual ACC, pgACC=pregenual ACC, INS=insula, Tpolar C=temporopolar cortex, AMY=amygdala. Row A shows regions of the brain that show greater rCBF in MDDart+ versus MDDart- patients (contrast A). A1: sagittal slice showing increased rCBF to the supragenual and pregenual ACC ($x=-2, y=26, z=24$), A2: coronal slice showing increased rCBF to the temporopolar cortex, bilaterally ($x=22, y=7, z=-21$), A3: sagittal slice showing increased rCBF to the right postcentral gyrus ($x=59, y=-25, z=34$). Row B shows regions of the brain that demonstrate reduced flow in the MDDart+ group relative to the MDDart- group (contrast A). B1: coronal image showing decreased rCBF to the right dorsal anterior insula/frontal operculum ($x=36, y=-1, z=15$), B2: axial slice showing decreased rCBF to the anterior insula, bilaterally ($x=36, y=-1, z=15$), B3: axial image showing decreased rCBF to the right anterior insula (located dorsal and rostral to the cluster of voxels activated in B2 in the vicinity of the intrasulcal BA47s cortex²⁰) ($x=26, y=20, z=6$) in the MDDart+ group relative to the MDDart- group. Row C shows regions of the brain associated with greater rCBF in response to arterial cannulation in the MDD+ group compared with the MDDart- group relative to differences between the HCart+ and HCart- groups (contrast C). C1: axial slice showing increased rCBF to the left temporopolar cortex and right amygdala (see figure 1 legend and below for amygdala coordinates), C2: coronal slice showing increased rCBF to the left temporopolar cortex and right amygdala ($x=24, y=3, z=-17$), C3: axial slice showing increased rCBF to the postcentral gyrus ($x=58, y=-24, z=34$). A4, B4, and C4: Key showing correspondence between image color and voxel t-value.

HCart+ versus HCart- subjects) in mesial and rostral temporal cortex, areas that share extensive anatomical connections with the amygdala and the medial prefrontal cortex, namely the right entorhinal cortex, the temporopolar cortex bilaterally, and the cortex along the rostral superior temporal sulcus bilaterally³³ (contrast A: table 4, fig. 2A and contrast C: table 6 and fig. 2C).

Depending on the type of stressor and the specific amygdala nucleus examined, chronic stress causes either dendritic atrophy or dendritic hypertrophy of amygdala neurons in

rodents³⁴, while in humans, structural MRI is suggestive of decreased amygdala volumes in unmedicated patients with bipolar depression³⁵. The functional correlates of this stress may be hyperexcitability of the basolateral amygdala (BLA) and bed nucleus of the stria terminalis (BNST)³⁶. While glucocorticoids usually act as a brake on hypothalamic CRF release, high levels of glucocorticoids potentiate the release of CRF from the BNST³⁷ and activate neurons in the BLA³⁸. This increase in excitability of the BLA has been shown to occur in a timeframe relevant to this study.



Table 6 | Whole brain analysis for contrast B showing regions where rCBF differed between HCart+ subjects versus HCart- subjects

Contrast B – Whole Brain Analysis								
Increased rCBF in HCart+ vs. HCart-								
Cluster Size	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region
					X	Y	Z	
23	3.67	3.58	<0.001	0.812	12	-16	63	R premotor cortex
Decreased rCBF in HCart+ vs. HCart-								
Cluster Size	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region
					X	Y	Z	
37	3.86	3.76	<0.001	0.928	-38	-41	-8	L parahippocampal gyrus

Significance threshold, abbreviations, and interpretation of the coordinates are as in table 2.

Duvarci and Pare showed that the principal neurons in the rat BLA increased their intrinsic activity within 1–2 hours of the administration of corticosterone³⁸. Given that we have previously shown arterial cannulation to induce cortisol production and given that cortisol increases amygdala excitability and CRF release, it is conceivable that the elevation in blood flow to the amygdala in the cannulated MDD patients but not the HCs, is indicative of an impaired neuroendocrine response to cannulation stress in the patients with MDD, such as an abnormal regulatory (negative feedback) response to cortisol secretion.

Henckens et al.³⁹ showed that administration of hydrocortisone to healthy men acutely diminished connectivity of the amygdala to both limbic structures such as the hippocampus and hypothalamus as well as connectivity of the amygdala to cortical structures such as the middle frontal gyri and middle temporal gyri. The authors suggest that this weakening of amygdala connectivity may be an adaptive measure to curtail the neuroendocrine stress response but that continuously elevated levels of circulating cortisol in MDD may result in a pattern of chronic amygdala decoupling³⁹. This hypothesis receives support from a study that reported that healthy volunteers showed a peak decrease in amygdala and hippocampal activity 25–30 minutes after intravenous administration of hydrocortisone⁴⁰. Since [¹⁵O]-H₂O PET imaging commenced approximately 30 minutes after arterial cannulation (see below), healthy volunteers, but not depressed subjects, may have been able to curtail their glucocorticoid-driven stress response in the time period between arterial cannulation and imaging. Thus, a disturbance of cortisol regulation may have contributed to the stress-associated increase in rCBF in the amygdalae of patients with MDD.

Regarding physical stressors, the amygdala shows increased neurophysiological response to pain in both healthy subjects⁴¹ and patients with MDD^{42,43}. Similarly, decreased sensitivity to pain in PTSD is associated with a reduction in the hemodynamic response of the amygdala⁴⁴. Of additional relevance is a recent study showing that inducing a sad mood in healthy subjects enhances pain sensitivity in response to a noxious stimulus. Greater subjective unpleasantness of the pain was correlated with activity in the amygdala bilaterally⁴⁵. Nonetheless we note that we cannot draw any conclusions about the degree of physical discomfort experienced by the MDD versus the HC groups in this study.

The positive correlation between rCBF in the right (and to a lesser extent, the left) amygdala is consistent with the finding of Abercrombie et al. who reported that regional cerebral metabolism in the right amygdala of depressed subjects was positively associated with negative affect measured using the Positive and Negative Affect Schedule (PANAS)⁴⁶. Our result is also consistent with our previous finding of a significant correlation between Hamilton Depression Rating scores and rCBF in the left amygdala in patients with MDD⁴⁷. Our study goes one step further by demonstrating that a physical stressor may induce this effect since the correlation between rCBF in the amygdala and depression severity was not observed in the MDDart- group.

- (3) A bilateral *increase* in rCBF was observed in the posterior insula (PI) in MDDart+ patients compared with MDDart- patients (contrast A: table 2, fig. 1A; contrast C: table 3, fig. 1). In contrast, *decreased* rCBF of the left and right dorsal anterior insula (AI)/ frontal operculum was observed in the MDDart+ versus the MDDart- group (contrast A: table 2, fig. 1B; table 4, fig. 2).

The insula is functionally and cytoarchitecturally differentiated along rostro-caudal lines⁴⁸. While the granular PI is

Table 7 | Whole brain analysis for contrast C showing regions of *increased* rCBF in the MDD sample versus the HC sample in the high versus low stress condition^a

CONTRAST C – Whole Brain Analysis								
Increased rCBF in MDD vs. HC								
Cluster Size	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region
					X	Y	Z	
38	3.66	3.57	<0.001	0.697	59	-25	34	R postcentral gyrus
44	3.64	3.55	<0.001	0.697	-34	5	-22	L temporopolar cortex
19	3.30	3.23	0.001	0.697	24	3	-17	R Amygdala

Significance threshold, abbreviations, and interpretation of the coordinates are as in table 2.

a. No region showed statistically significant decreases in rCBF for contrast C.



Table 8 | Whole Brain Analysis showing regions of increased rCBF in the MDD sample versus the HC sample in the low stress condition (contrast D)^a

CONTRAST D – Whole Brain Analysis								
Increased rCBF in MDDart- vs. HCart-								
Cluster Size	T score	Z score	p-value	FDR p-value	Stereotaxic Coordinates			Region
					X	Y	Z	
14	3.41	3.34	<0.001	0.972	29	-32	4	R Posterior Hippocampus

Significance threshold, abbreviations, and interpretation of the coordinates are as in table 2.
a. No regions showed statistically significant decreases in rCBF for contrast D.

closely linked to the somatosensory cortex and parietal operculum, and provides an interoceptive map of the physiological state of the body, the agranular AI is thought to provide more abstract emotional information that flows from this corporeal representation. Specifically, Craig⁴⁸ postulated that sensory information from the PI is initially re-represented in the ipsilateral AI, but then undergoes a second-order remapping in the right AI, allowing for the subjective emotional experience associated with physiological arousal/stress. That is, the AI appears to play a role in translating interoceptive states into subjective feelings.

Consistent with this hypothesis, the AI is activated not only by aversive physical stressors but also by “noxious” psychological stimuli such as watching a loved one in pain⁴⁹, imagining oneself to be in pain⁵⁰ or believing that one is being exposed to harmful mobile phone radiation⁵¹. Greater activation of the right AI is also observed in response to painful stimulation after sad mood induction⁵².

Since all of these studies reporting increased activity in the AI in response to noxious stimuli were conducted on healthy volunteers, it could be argued that the decrease in AI blood flow observed in our MDDart+ sample compared with the MDDart- sample is reflective of an abnormal insular response to physiological stress in MDD. This hypothesis is consistent with the finding of Brody et al.⁵³ who reported that MDD patients showed increases in glucose metabolism in the left anterior insula (x y z coordinates: -46, 20, 8 versus a secondary peak in our study at -40, 18, 6) after treatment with interpersonal psychotherapy. (Nevertheless, it should be noted that these coordinates actually localize to the frontal operculum associated with the inferior frontal gyrus, in the stereotaxic atlases of Mai et al.⁵⁴ and Talairach and Tournoux⁵⁵ - see discussion, below). Similarly, a recent meta-analysis of the MDD literature reports decreased activity of the left AI (-36, 7, 4) during exposure to negative stimuli⁵⁶. Conceivably, the discrepant findings regarding functional activity of the AI in depressed subjects as opposed to healthy controls also may be related to HPA axis function. Hubbard et al.³² found that compared with healthy controls, anxious IBS patients displayed a strong negative correlation between the resting BOLD response of the AI and the amygdala during expectation of abdominal pain. However, after administration of a CRF antagonist, the negative coupling between the AI and the amygdala was attenuated.

In contrast, other studies of depressed patients with BD have demonstrated increased glucose metabolism of the right AI⁵⁷ (34, 2, 2) increased rCBF in the right AI after induction of sadness^{58,59} (38, 4, 0 and 44, 16, 4), and a decrease in glucose metabolism of the both the AI and PI after successful treatment with paroxetine⁶⁰ (-36, 2, 0; 40, 22, -2) and fluoxetine⁶¹ (-22, 22, 6 and 36, 14, 8). Similarly, MDD patients have been reported to show post-treatment increases in glucose metabolism in the ventral AI (30, 18, -8 and -16, 18, -16) and the

midanterior AI (30, -2, 8 and -26, -4, 8)⁶². Consistent with these data, increased activation of the PI, bilaterally, and the left AI (-24, 24, 6) in response to noxious heat stimulation in MDD was reported⁶³. Moreover Strigo et al.⁴³ reported that during anticipation of painful stimuli relative to non-painful stimuli depressed subjects displayed greater activation than controls in the right AI, although the region implicated actually localized to the inferior frontal gyrus (44, 22, 9).

While most studies show acutely increased activity of the AI in response to negative stimuli, one possible explanation for the decrease in rCBF in the AI in the MDDart+ versus the MDDart- group is that the decrease in AI activity is indicative of impaired sensory integration under stress. Strigo et al. reported that compared with healthy controls, unmedicated patients with MDD showed a decreased BOLD response in the vicinity of the right AI located in lateral orbital cortex (BA47; Ongur et al.²⁰) (37, 24, -7) in response to cues predicting a shift in the intensity of a stimulus from non-painful warmth to painful heat and vice versa⁶⁴. This cortex forms part of the “orbital” or “sensory” prefrontal network described by Ongur et al.⁶⁵ which plays a major role in sensory integration. Strigo et al. speculated that this deficit prevents the MDD patients from attributing accurate emotional value to physiological safety cues⁶⁴. Interestingly, the AI plays a role in fear extinction. At least 3 studies have reported *increased* activity in the AI during extinction learning in *healthy* individuals^{66,67}. Gottfried and Dolan reported neural activation of the AI (39, 12, 9 and -33, 15, 12) during the extinction-learning phase of an olfactory conditioning task⁶⁶, while hemodynamic response of the AI (-35, 14, 3 and 31, 20, 5) was associated with extinction to conditioned shock responses⁶⁸. In a more recent study, volunteers showed an increased hemodynamic response in the vicinity of the AI, located in the frontal operculum (-40, 20, 2 and 40, 20, -2) but a decreased hemodynamic response in the amygdala during the extinction phase of a cued fear-conditioning task⁶⁷. Conceivably, the changes in rCBF in the AI reported here may be related to the suboptimal extinction of fear responses in patients with mood and anxiety disorders⁶⁹.

In sum, the literature on insula function in depression is confusing perhaps because the insula is a large and heterogeneous brain region with a highly complex pattern of internal and external connections. The studies that have implicated the AI in depression have generally reported increased activity in a more ventral region of the AI than reported here and this may be one reason for the discrepancy of our data with some of these aforementioned reports. The coordinates of our peak voxels (tables 2 and 5), are not centered in a region that is classically thought to be agranular AI. Rather according to the anatomical atlases of Mai et al.⁵⁴ and Talairach and Tournoux⁵⁵, the peak voxels in our study are found in the dorsal insula/frontal operculum and in BA47s (Walker 12o in the monkey)²⁰. Our group has recently shown the dorsal

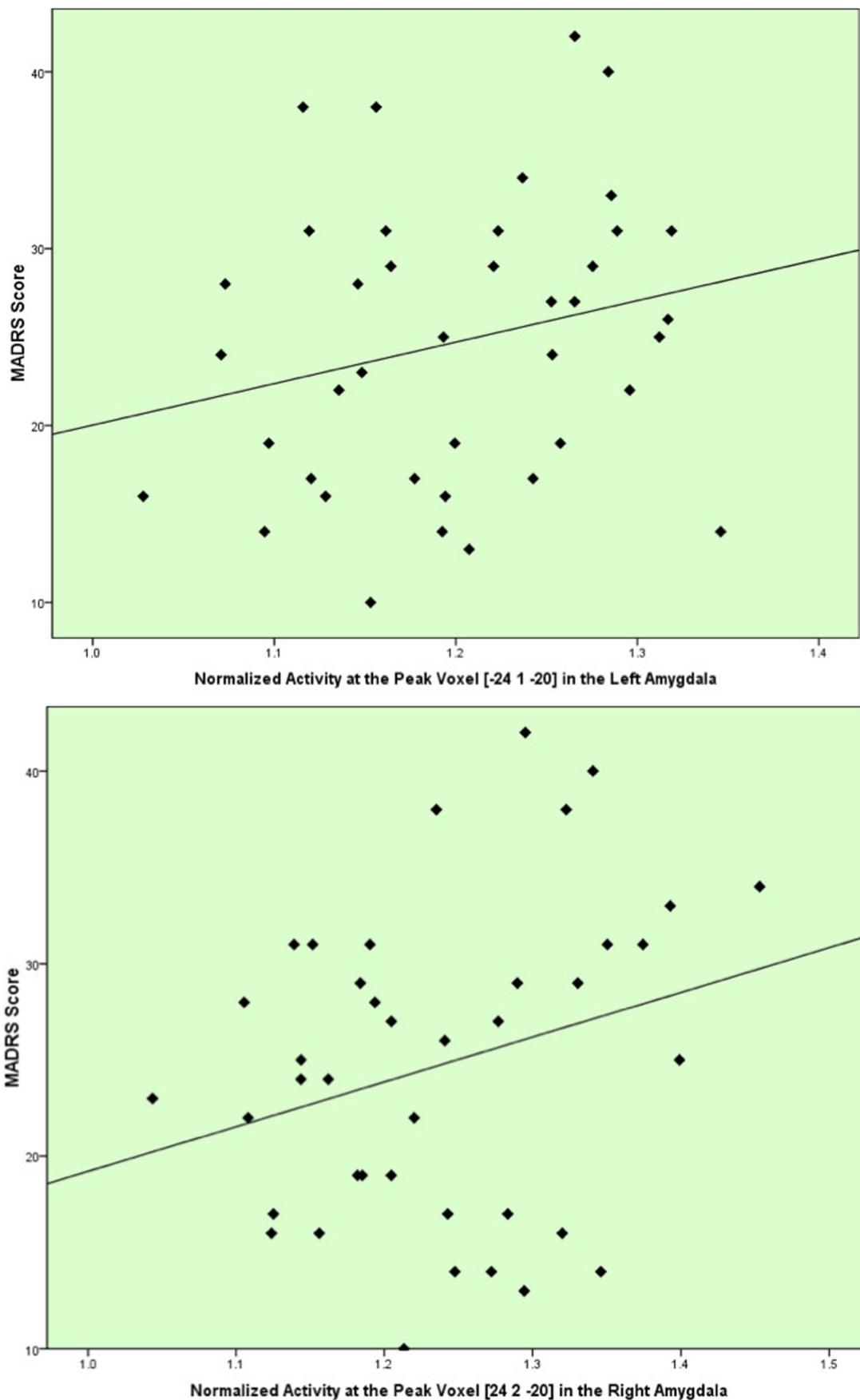


Figure 3 | Correlations between the MADRS scores and the normalized activity at the peak voxels in the left amygdala [$x=-24, y=1, z=-20$] and the right amygdala [$24, 2, -20$] in the MDDart+ group. The peak voxel for the left amygdala was obtained from the ROI analyses (contrast A, table 2, contrast C, table 3). Note: The normalized activity is unitless because it is a ratio of activity at the peak voxel to mean activity over the whole brain. The solid line represents the line of best fit for the data.



insula/frontal operculum to be activated specifically in response to interoceptive (as opposed to exteroceptive) stimuli at almost identical stereotaxic coordinates to those reported here ($-37, -3, 16$ versus $36, -1, 15$ and $37, -3, 16$ versus $-40, -7, 17$)⁷⁰. Moreover Avery et al. reported that compared with healthy volunteers, depressed MDD subjects showed a reduction in BOLD activity in response to the eliciting of interoceptive stimuli (stomach, heart, and bladder) that was correlated with depression severity⁷⁰. Clearly, the presence of an arterial cannula is a salient interoceptive stimulus. Conceivably, patients with MDD are impaired at assigning emotional value (including physiological safety cues)⁶⁴ to interoceptive stimuli, thus accounting for the decrease in rCBF in the dorsal AI in the MDDart+ versus the MDDart-groups, but not in the HCart+ versus the HCart- groups.

- (4) The MDDart+ patients displayed *decreased* rCBF compared with MDDart- patients in the sgACC (contrast A: table 2, fig. 2A).

The sgACC has been implicated in pain as well as mood disorders⁷¹. While increased sgACC activity is usually associated with MDD⁷¹, increased activity of this region is also associated with pain relief. For example, activation of the sgACC has been reported in conjunction with placebo-induced analgesia⁷², deep brain stimulation-induced relief of phantom limb pain⁷³ and habituation to experimentally-induced pain⁷⁴. On the other hand, Lopez-Sola⁶³ found that compared with healthy controls, MDD patients failed to deactivate the sgACC and the pregenual ACC during painful stimulation. Conceivably, the cannulation-associated decrease in rCBF in the sgACC in our sample of individuals with MDD, is reflective of a disturbance in visceromotor network homeostasis which retards extinction of somatic arousal and cannulation-associated stress. This hypothesis is congruent with a recent report that examined the neural correlates of courage as indicated by the ability to overcome fear in people with snake phobia⁷⁵. Allowing a live snake to be brought closer to their heads without succumbing to fear was associated with increased hemodynamic response of the sgACC together with decreased activity of the amygdala. Moreover, the increase in sgACC activity was associated with a decrease in somatic arousal as measured by skin conductance responses.

- (5) Increased rCBF in the right somatosensory cortex and precuneus was observed in the whole brain analysis of the MDD group in the high versus low stress condition (contrast A: table 4, fig. 2A and contrast C: table 6, fig. 2C).

In the meta-analysis of Apkarian et al.¹⁸ 46 out of 68 studies of healthy subjects showed significant changes in activity of the somatosensory cortex during pain induction. The cluster of increased rCBF in the right postcentral gyrus is consistent with left-sided arterial cannulation. Somatosensory cortex I (SI) has a somatotopic arrangement and is involved in localization of pain on the body surface. SII, however, contains fewer nociceptive neurons and may play more of a role in spatially directed attention towards the painful stimulus rather than sensory-mechanical discrimination of the stimulus⁷⁶. SII may also play a role in the processing of more abstracted pain stimuli. SII is activated by vicarious observation of trauma⁷⁷ and hypnosis-induced pain⁷⁸. In fact, a recent study even reported activation of SI during the processing of pain-related words⁷⁹. Finally, the posterior insula, which also showed increased rCBF in MDDart+ patients compared with MDDart- patients, contains a major sensory association cortex⁸⁰.

Thus, the increased arterial cannulation-associated rCBF of the right postcentral gyrus in our MDD group is consistent with 3 non-mutually exclusive possibilities: increased physical sensitivity to the invasive stimulus in MDD, a heightened attentional

awareness of the arterial line in our MDD sample compared with the HC sample, and greater physiological stressor-driven emotional processing in MDD. The higher rCBF in the somatosensory cortices in the postcentral gyrus in MDD subjects versus controls under arterial cannulation stress appears particularly noteworthy in light of the “somatic marker hypothesis” of emotion proposed by Damasio and his colleagues. This hypothesis proposes that the visceral or bodily reaction that normally accompanies emotion or unease (the somatic marker) is experienced in a network subserved by both the somatosensory cortices and portions of the ventromedial prefrontal cortex that include the perigenual ACC and medial orbitofrontal cortex⁸¹. If so, then over-activity of this system in MDD in response to stressors may hypothetically produce the chronic sense of “unease” that is a frequent component of depression.

Similarly, the precuneus has been associated with pain processing in a number of recent imaging studies perhaps because of its role in self-referential processing⁸². The default mode network (DMN) refers to regions of the brain that are thought to mediate self-referential processing, are most active when the brain is in a resting state, and that are largely suspended when the individual is actively engaged in effortful cognitive tasks⁸³. DMN regions include the dorsal and ventral mPFC, posterior cingulate/precuneus, parietal cortex, and the superior temporal gyrus (STG)⁸³. These regions have been demonstrated to be inappropriately active in patients with MDD, leading Sheline et al.⁸⁴ to hypothesize that patients with MDD have an impaired ability to shift their attention away from the internal milieu to meet external cognitive demands, and others to account for depression-related rumination with this model⁸⁵.

If correct, the DMN model not only explains the salience of negative emotion in MDD but raises the possibility that disproportionate internal bodily surveillance increases the intensity or unpleasantness of somatic signals in MDD. Consistent with this suggestion, chronic pain patients demonstrate abnormally elevated BOLD activity in the DMN when performing a simple visual attention task⁸⁶ and healthy subjects exposed to noxious stimuli show connectivity changes in the posterior cingulate cortex and the precuneus⁸⁷. Thus the increase in rCBF in the ACC, inferior parietal lobe, precuneus, and STG in the MDDart+ sample compared with the MDDart- sample, but not in the HCart+ sample compared with the HCart- sample, is consistent with the evidence for a proclivity towards higher activity of the DMN in MDD; a predisposition that may be instantiated under conditions of physiological stress.

- (6) The MDDart- group showed increased rCBF in the posterior hippocampus compared with the HCart- group. A reduction in hippocampal volume is one of the most widely reported findings in MDD^{88–90}. We have previously suggested that this MDD-associated reduction in hippocampal volume is more pronounced in the posterior hippocampus than the anterior hippocampus. Our group reported a reduction in posterior but not anterior hippocampal volume in unmedicated, remitted patients with MDD compared with healthy controls⁹¹, as well as a reduction in posterior hippocampal volume in patients with post traumatic stress disorder (PTSD) versus healthy controls⁹². Approximately half of the PTSD group in this study had co-morbid MDD.

The rodent analogue of the human posterior hippocampus is the dorsal hippocampus. Compared with other hippocampal regions, the CA1 neurons in the dorsal hippocampus have been demonstrated to be particularly sensitive to ischemia⁹³, possibly because of the dense concentration of NMDA receptors in this region⁹⁴, which may confer vulnerability to glutamate-induced excitotoxicity. To our knowledge this is the first report of elevated rCBF specifically in the *posterior* hip-



pocampus in depressed patients versus healthy controls, and provides support to our previous neuromorphometric findings in depressed populations.

- (7) In contradistinction to the widespread differences in rCBF that were found between the MDDart+ and MDDart- groups in both the ROI and whole brain analyses (tables 2 and 5), the HCart+ and HCart- groups did not show differences in rCBF in the ROI analysis, and showed only modest differences in rCBF in the whole brain analysis (table 6). These data potentially support the conclusion that the functional anatomical responses to physiological stress are exaggerated in individuals with MDD.

There is increasing evidence of an overlap in the neural circuits that underlie physical and social pain/stress⁹⁵. Our results thus raise the possibility that individuals who are neurophysiologically resilient to physical stressors may also be resilient to psychological stressors. Conceivably, future research could be aimed at evaluating the feasibility of using the neurophysiological response to a physical stressor as a marker of vulnerability to mood disorders.

A number of limitations deserve mention. This study was not designed to specifically induce pain in participants. We therefore did not administer psychometric instruments to measure pain after arterial catheterization. Thus, although our MDD patients appeared more *neurophysiologically* sensitive to the stressor of arterial cannulation than HC subjects, we cannot draw any conclusions about differences in their *psychological* response to arterial cannulation with respect to the subjective experience of pain or distress. Moreover, the subjective experience of arterial cannulation varies across subjects, such that some individuals report more pain and discomfort than others. Arterial cannulation was performed approximately 30 minutes prior to the scan. Thus the initial discomfort experienced by subjects may have subsided prior to the commencement of scanning. We note, however, that glucocorticoids exert both rapid non-genomic, and slow genomic effects, both of which persist for a number of hours⁹⁶. Henckens et al.⁹⁷ recently reported that administration of hydrocortisone to healthy individuals 75 minutes prior to fMRI scanning modulated amygdala activity. If the neurophysiological changes associated with arterial cannulation reported herein were at least partly related to glucocorticoid function, then MDD patients with and without glucocorticoid resistance would be expected to differ in their neurophysiological response to cannulation. The study would therefore have been strengthened by the administration of endocrine assays that would have allowed us to differentiate between MDD patients with and without glucocorticoid resistance.

Our study was cross-sectional rather than longitudinal in nature and therefore despite the fact that inclusion and exclusion criteria as well as severity of depression was indistinguishable across cannulation versus non-arterial cannulation studies, it is possible that the art+ and art- groups differed on unmeasured clinical or genetic traits that accounted for the results. The results reported here might not be generalizable to other types of stress. For instance, different results may have been obtained with exposure to transient, superficial pain which is commonly used in most experimental manipulation, is possibly less unpleasant than deeper, visceral pain⁹⁸, and may provoke less of an affective response in PET studies⁹⁹.

Finally, although our sample is very large for a PET imaging study the statistical sensitivity of our results was limited by the relatively small sample sizes of the non-cannulated samples. This reduced our power to detect differences between the participants who underwent arterial cannulation relative to those who did not, and reduced power to detect differential

sensitivity to arterial cannulation stress between MDD subjects and healthy controls in contrast C.

In conclusion, we raise the possibility that MDD patients are more sensitive – at least neurophysiologically – to invasive physical stressors than healthy individuals. This difference conceivably may account for the increased incidence of somatic-related complaints in MDD. Perhaps visceromotor regions such as the ACC and insula, which evolved to process somatic pain, have been co-opted to process negative emotions. Thus on the neurobiological level, exposure to emotional pain and independent physical pain, may be akin to a “double-dose” of either emotional or physical pain. Further exploration of response to physiological stressors in MDD may enhance our understanding of the neurobiology of emotion.

Methods

Participants. The participants were drawn from several different PET neuroreceptor mapping studies, all of which were approved by the NIMH Institutional Review Board. All subjects provided written informed consent to participate. Detailed demographic and clinical data appear in table 1.

Sixty-six individuals with MDD and 79 healthy controls participated in PET studies at the NIH. Blood flow images were acquired using [¹⁵O]-H₂O before neuroreceptor imaging studies that employed a range of radioligands, only some of which required an arterial input function for tracer kinetic analysis. Thus 51 MDD patients completed [¹⁵O]-H₂O scanning with arterial cannulation (MDD art+) and 15 MDD patients completed [¹⁵O]-H₂O scanning without arterial cannulation (MDD art-). Sixty-two HC subjects completed [¹⁵O]-H₂O scanning with arterial cannulation (HC art+) and 17 HC subjects completed [¹⁵O]-H₂O scanning without arterial cannulation (HC art-).

Participants met DSM-IV criteria for MDD based on the Structured Clinical Interview for DSM-IV¹⁰⁰ and an unstructured interview with a psychiatrist. The following exclusion criteria applied: left-handedness, history of bipolar disorder, treatment with psychiatric medication within 3 weeks of scanning (6 for fluoxetine), cigarette smoking, significant medical or neurological disorders, past head injury with loss of consciousness, serious risk of suicide, meeting DSM-IV criteria for substance abuse within the previous 6 months or substance dependence within the previous 5 years, general MRI exclusion criteria, electrolyte disturbance, anemia, or positive drug, hepatitis or HIV screen. HC subjects had no lifetime history of a psychiatric disorder, and no first degree relative with a mood or anxiety disorder, as measured by the Family Interview for Genetic Studies (FIGS). There were no differences in inclusion/exclusion criteria for subjects receiving arterial cannulation versus those not receiving arterial cannulation.

Twenty individuals in the MDD art+ group were medication naive and the remainder had been unmedicated for an average of 93 ± 120 weeks. Ten individuals in the MDD art- group were medication naive and the remaining subjects were unmedicated for an average of 28 ± 9.0 weeks. This difference in time spent unmedicated was not statistically significant (table 1).

Eight individuals in the MDD art+ group and 2 individuals in the MDD art- group had a remote history of substance abuse (alcohol or cannabis). No individuals had a history of substance dependence. Comorbid disorders present in the MDD art+ group included panic disorder (PD) (n=5), post-traumatic stress disorder (PTSD) (n=4), other anxiety disorders (n=3), and eating disorders (n=2). Comorbid disorders found in the MDD art- group included PD (n=3), PTSD (n=4), other anxiety disorders (n=2), and eating disorders (n=1).

Depression severity was rated using the Montgomery-Asberg Depression Rating Scale (MADRS). Mean MADRS scores of the MDD art+ and MDD art- groups were 25 ± 8.1 and 23 ± 5.4, respectively.

Positron Emission Tomography. The MDD art+ and HC art+ participants underwent right antecubital venous cannulation and left radial artery cannulation prior to PET imaging. The latter procedure was performed by an anesthesiologist in the post-anesthesia care unit of the NIH Clinical Center approximately 30 minutes prior to commencement of the PET scan. EMLA cream was applied and a local anesthetic was injected into the wrist prior to arterial catheterization. The MDD art- and the HC art- subjects also underwent right antecubital venous cannulation 30–60 minutes prior to the start of the PET scan.

Each subject underwent two 40-second emission scans while resting with eyes closed after bolus IV injection of 10 mCi of [¹⁵O]-H₂O.

Images were acquired in 3D mode on a GE Advance scanner and reconstructed to a 3D spatial resolution of 6.5 mm full-width-at-half-maximum. Corrections included measured attenuation. The [¹⁵O]-H₂O arterial time activity curve was measured with an automated blood counting system which sampled blood at 3.8 ml/min during the [¹⁵O]-H₂O scans in the cases who had arterial cannulae. So that the image data remained comparable between the subjects with versus without arterial input functions, the images of regional tissue radioactivity concentration, which are proportional to rCBF⁹, were compared after normalizing the data to exclude nonspecific global effects on tissue [¹⁵O]-H₂O uptake.



Regions of Interest. We expected to observe MDD-associated changes in rCBF in the high-stress condition in the amygdala, ACC and insula. These regions were identified using the Wake Forest University PickAtlas. The ROI were used to constrain the voxel-wise analysis using the Small Volume Correction option within SPM5.

Statistical Analysis. Analysis was carried out using SPM5. The two [15 O]-H $_2$ O scans were averaged (in order to improve signal-to-noise ratio) and coregistered to a whole brain MR image acquired using an MP-RAGE sequence (TE=4.94 msec; TR=11.6 msec, prep time=725 msec; delay time=1400 msec; voxel size=0.85×0.85×1.2 mm). After coregistration, PET images were spatially normalized to the standard T1 template and smoothed using an 8 mm Gaussian kernel.

Analysis was performed using a general linear model: a 2×2 ANOVA with diagnosis (MDD versus HC) and arterial line (yes versus no – that is, high versus low stress condition) as main factors, and age as a covariate. Results were normalized by global activity using the SPM5 default grand-mean scaling value of 50.

In order to measure if arterial cannulation differentially affected the rCBF of MDD and HC subjects, we subtracted the effects of arterial cannulation in the HC group (HCart+ – HCart-) from the effects of arterial cannulation in the MDD group (MDDart+ – MDDart-). In mathematical terms: [(MDDart+ – MDDart-) – (HCart+ – HCart-)]. We have labeled these contrasts as A-B=C. In addition, we contrasted the MDDart- vs. the HCart- group in order to evaluate group differences in rCBF that were specifically related to diagnosis (contrast D).

For the ROI analyses the coordinates for peak voxel t-values exceeding a threshold of $p < 0.001$ were reported if they were located within a cluster of contiguous voxels for which $p \leq 0.01$ that was at least 100 mm 3 in size (13 voxels). To reduce Type II error whole brain analyses were performed *post hoc* to locate differences in CBF located outside the regions of primary interest. For the whole brain analysis, the coordinates for peak voxel t-values exceeding $p < 0.001$ were reported if they were located within a cluster of contiguous voxels for which $p \leq 0.001$ that was at least 100 mm 3 in size.

In order to test whether the severity of depressive symptoms affected rCBF in MDD patients, we correlated the activity at the peak voxel of each cluster reaching statistical significance in contrast C (interaction) with the MADRS score for that individual. Specifically, the ROI analysis yielded two significant clusters with peak voxels in the right amygdala [24, 2, -20] and supragenual ACC [0, 27, 28]. Three clusters in the right postcentral gyrus [59, -25, 34], left temporopolar cortex [-34, 5, -22] and right amygdala [24, 3, -17] reached statistical significance in contrast C in the whole brain analysis. However, the activity at the voxel at coordinate [24, 3, -17] in the right amygdala was not evaluated for correlation with the MADRS scores because of its close proximity to the peak voxel coordinate in the ROI analysis [24, 2, -24], which would have rendered the analysis redundant. We previously reported an association between glucose metabolism in the left amygdala and depression severity⁴⁷, and therefore we also conducted a correlation analysis with the peak voxel the left amygdala that reached statistical significance in contrast A [-24, 1, -20]. Because of the *a priori* evidence for a positive association between amygdala activity and severity of depression, we conducted the correlation analyses involving the left and right amygdala with a one-tailed Pearson's correlation coefficient test. All other correlations were conducted using a two-tailed Pearson's test.

The distribution of gender across the 4 subject groups was measured using the chi-squared test (two-tailed, $p < 0.05$). An ANOVA was used to test for differences in age across the 4 groups (two-tailed, $p < 0.05$). Independent sample t-tests (two-tailed $p < 0.05$) were used to compare the MDD art+ and MDD art- groups on the following variables: number of weeks medication free, age at illness-onset, duration of illness, and MADRS, scores.

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Author contributions

JS wrote the paper and conducted the imaging and statistical analyses, ACN assisted with data analysis, DMC scanned subjects, PJC scanned subjects, RD assisted with data analysis, AN scanned subjects, DRS assisted with data acquisition and provided clinical support, SF assisted with data analysis, PH runs the

PET center and developed the methods for [^{15}O]- H_2O analysis, WCD designed the study and assisted with the writing of the manuscript. All authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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