Archival Report

Biological Psychiatry

Striatal Hypersensitivity During Stress in Remitted Individuals with Recurrent Depression

Roee Admon, Laura M. Holsen, Harlyn Aizley, Anne Remington, Susan Whitfield-Gabrieli, Jill M. Goldstein, and Diego A. Pizzagalli

ABSTRACT

BACKGROUND: Increased sensitivity to stress and dysfunctional reward processing are two primary characteristics of major depressive disorder (MDD) that may persist after remission. Preclinical work has established the pivotal role of the striatum in mediating both stress and reward responses. Human neuroimaging studies have corroborated these preclinical findings and highlighted striatal dysfunction in MDD in response to reward but have yet to investigate striatal function during stress, in particular in individuals with recurrent depression.

METHODS: A validated mild psychological stress task involving viewing of negative stimuli during functional magnetic resonance imaging was conducted in 33 remitted individuals with a history of recurrent major depressive disorder (rMDD) and 35 matched healthy control subjects. Cortisol and anxiety levels were assessed throughout scanning. Stress-related activation was investigated in three striatal regions: caudate, nucleus accumbens, and putamen. Psychophysiologic interaction analyses probed connectivity of regions with central structures of the neural stress circuitry, such as the amygdala and hippocampus.

RESULTS: The task increased cortisol and anxiety levels, although to a greater extent in rMDD individuals than healthy control subjects. In response to the negative stimuli, rMDD individuals, but not controls, also exhibited significantly potentiated caudate, nucleus accumbens, and putamen activations and increased caudate-amygdala and caudate-hippocampus connectivity.

CONCLUSIONS: The findings highlight striatal hypersensitivity in response to a mild psychological stress in rMDD, as manifested by hyperactivation and hyperconnectivity with the amygdala and hippocampus. Striatal hypersensitivity during stress might thus constitute a trait mark of depression, providing a potential neural substrate for the interaction between stress and reward dysfunction in MDD.

Keywords: Caudate, Depression, fMRI, Psychophysiologic Interaction (PPI), Reward, Stress

http://dx.doi.org/10.1016/j.biopsych.2014.09.019

Major depressive disorder (MDD) is a highly recurrent psychiatric condition and thus a significant public health problem (1). According to the "kindling/sensitization" theory, recurrence of depression may stem from sensitization of the stress response, rendering remitted individuals particularly susceptible to the effects of minor daily stressors (2,3). Indeed, stress was found to be a robust predictor of depression relapse rates (4). In addition to increased stress susceptibility, remitted individuals continue to exhibit reduced response to positive stimuli, a cardinal symptom of MDD (5,6). Critically, animal (7–11) and human (12–16) studies provided converging evidence that stress can disrupt behavioral responses to rewards, suggesting that dysfunctional interactions between stress and reward may underlie anhedonia and MDD (17).

Extensive preclinical evidence has established the mediating role of the ventral (i.e., nucleus accumbens [Nacc]) and dorsal (i.e., caudate, putamen) striatum in both reward and stress processing (18,19), raising the possibility that the striatum might be a structure in which stress and reward processing interact. Specifically, electrophysiologic studies in nonhuman primates showed that striatal (and midbrain) dopamine (DA) signaling track reward-related prediction errors (20–22), whereas stressors (e.g., foot shock, social defeat) were shown to elicit robust DA release in the rat striatum and medial prefrontal cortex (23–25). Findings from human neuroimaging studies have corroborated the key role of the striatum within the reward circuitry (26,27), and abnormal striatal responses have been described that might account for dysregulated reward processing in current (28–34) and remitted (35,36) MDD. However, less attention has been devoted to striatal function in humans during acute stress, particularly in individuals with recurrent depressive episodes. Most of the human neuroimaging stress literature focuses on the amygdala and hippocampus as pivotal mediators of the stress response (37,38) and its regulation (39). Along those lines, individuals with current MDD (40–43) and remitted individuals (44–48) exhibited hyperactive amygdala and hippocampus in response to negative affective stimuli and stress, including in a subgroup of female subjects from the present sample (49).

ISSN: 0006-3223

To fill this gap in the literature, we evaluated activation and connectivity of striatal regions during stress in healthy and remitted individuals with recurrent major depressive disorder (rMDD). In the context of the kindling/sensitization theory, suggesting that remitted individuals are particularly susceptible to the effects of minor stressors (2,3), we exposed 32 remitted individuals with a history of rMDD and 35 matched healthy control subjects to a mild psychological stress task during functional magnetic resonance imaging (fMRI), focusing on striatal activation. Furthermore, psychophysiologic interaction (PPI) (50–52) connectivity analyses were performed to investigate stress-specific changes in striatal connectivity with core stress circuitry regions, such as the amygdala and hippocampus. In light of 1) stress hypersensitivity in rMDD, 2) hyperactivity in amygdala and hippocampus in response to negative stimuli in rMDD (44–49), and 3) preclinical evidence indicating that acute stressors elicit robust DA release in striatum (23–25), which has been linked to increased fMRI responses (53), we hypothesized that during stress the rMDD group would exhibit increased striatal activation and increased connectivity with the amygdala and hippocampus.

METHODS AND MATERIALS

Participants

Participants were offspring of women who took part in the large (N = 17,741) Boston and Providence Collaborative Perinatal Project, also known as the New England Family Study (54). Structured Clinical Interview for DSM performed in a subsample of these offspring identified 205 individuals with a diagnosis of recurrent episodes of MDD and 706 healthy control individuals. From this group, 33 individuals with a diagnosis of rMDD were recruited for neuroimaging based on current mood status and magnetic resonance imaging eligibility criteria. Remission was defined as not meeting DSM-IV-R criteria for MDD for 30 days before scanning. In addition, on the morning of the study visit, participants in the rMDD group completed the 17-item Hamilton Depression Rating Scale ($HAM\text{-}D_{17}$) (55) to assess depressive symptoms (Table 1). Among the rMDD group, $HAM\text{-}D_{17}$ scores ranged from 0 (reported by n = 13 [39.4%]) to 10 (reported by n = 2 [6.1%]). Full remission was confirmed in 27 of the 33 rMDD individuals (81.2%) who had a $HAM\text{-}D_{17}$ score ≤7. The mean $HAM\text{-}D_{17}$ score was 4.2 (SD = 4.2).

We also recruited 35 healthy control subjects matched with regard to sex, ethnicity, handedness, parental socioeconomic status (SES), education, general intelligence, and mean menstrual cycle day for female subjects (Table 1). No woman was taking oral contraceptives or hormone replacement therapy or was menopausal. At the time of the study, 13 participants of the rMDD group were taking psychotropic medication, and no participants in the control group were taking psychotropic medication (Table 1). Comorbid current or past Axis I diagnoses are also reported in Table 1. Given that the rMDD group was slightly older than the control group, participants' age and SES were added as covariates in all analyses. Participants received payment for their time and provided written informed consent to a protocol approved by the Committee on the Use

Table 1. Demographic and Clinical Characteristics of Remitted Individuals with a History of rMDD and Healthy Control Subjects

	Healthy Control Group (n = 35)		rMDD Group (n = 33)	
Characteristic	Mean	SD	Mean	SD
Age (Years)[a]	45.7	2.7	47.4	1.8
Parental SES[b]	6.2	1.8	5.7	1.9
Education (Years)	14.5	2.4	13.5	2.0
Estimated Full Scale IQ[c,d]	110.7	13.5	107.6	12.5
Age at Onset of Major Depression (Years)	—	—	24.5	8.8
Duration of Illness (Years)	—	—	21.6	9.3
Number of Prior Major Depressive Episodes	—	—	5.0	2.3
Duration of Remission (Years)	—	—	7.3	6.3
Hamilton Depression Rating Scale (17-Item)	—	—	4.2	4.2
	No.	%	No.	%
Female	16	45.7	17	51.5
Caucasian	35	100	33	100
Handedness (Right)[d]	34	97.1	32	96.9
Current Psychotropic Medication[e]	—	—	13	39.4
Comorbid Diagnosis				
Current[f]	—	—	18	54.6
Past[g]	11	30.6	24	72.7

rMDD, recurrent major depressive disorder; SES, socioeconomic status.

[a]Significant difference between groups ($p < .05$).

[b]Parental SES was a composite index of family income, education, and occupation and ranged from .0 (low) to 9.5 (high).

[c]Full Scale IQ estimated using the sum of age-scaled scores from the Wechsler Adult Intelligence Scale–Revised Vocabulary and Block Design subtests and the conversion table C-37 from Sattler JM (1992): Assessment of Children, 3rd ed. San Diego: Jerome M. Sattler, 851.

[d]Data missing for one subject from the rMDD group and one subject from the HC group.

[e]In the rMDD group, 13 subjects (6 men) were currently taking the following medications: fluoxetine (n = 3); citalopram (n = 2); citalopram + alprazolam (n = 1); duloxetine + trazodone (n = 1); fluoxetine + clonazepam (n = 1); clozapine (n = 1); quetiapine (n = 1); sertraline + methylphenidate + clomipramine + gabapentin (n = 1); sertraline + buproprion (n = 1); venlafaxine + bupropion (n = 1).

[f]Current comorbid Axis I diagnoses in the rMDD group included two subjects with dysthymic disorder; two subjects with obsessive-compulsive disorder; three subjects with anxiety disorder, not otherwise specified; one subject with posttraumatic stress disorder; five subjects with panic disorder, without agoraphobia; two subjects with attention-deficit/hyperactivity disorder, not otherwise specified; one subject with alcohol dependence; and two subjects with social phobia.

[g]Past comorbid Axis I diagnoses in the rMDD group included two subjects with panic disorder, with agoraphobia; three subjects with alcohol dependence; nine subjects with alcohol abuse; two subjects with cocaine dependence; one subject with cannabis dependence; three subjects with cannabis abuse; two subjects with opioid dependence; one subject with sedative dependence; and one subject with anxiety disorder, not otherwise specified. In the healthy control group, past Axis I diagnoses included one subject with dysthymic disorder, two subjects with alcohol dependence, one subject with uncomplicated alcohol withdrawal, two subjects with cannabis abuse, one subject with hallucinogen abuse, three subjects with alcohol abuse, and one subject with caffeine-induced anxiety disorder.

of Human Subjects in Research at Harvard University and Brown University.

Stress Task

The task and its ability to evoke mild psychological stress response have been described and validated in multiple studies and populations (49,56–58). Briefly, 144 International Affective Picture System images were selected and sorted into two sets, one of negative valence/high arousal and the other of neutral valence/low arousal. A set of fixation images was created by applying Fourier transforms on the neutral valence/low arousal images. Each image was presented for 5 sec within a 30-sec block consisting of six images of unified content (negative/high arousal or neutral/low arousal or fixation). For each content, 12 blocks were presented during scanning in a counterbalanced order, yielding three 6-min functional scans. To maintain attention to the stimuli, participants were asked to press a button each time the picture changed, regardless of its content.

Stress Response Assessment

After the scan, participants rated the negative and neutral images for arousal and valence using Self-Assessment Manikin scales (59). Anxiety levels before and after scanning were assessed using the state form of the Spielberger State-Trait Anxiety Inventory (60). Cortisol stress response was assessed using serial blood samples collected during fMRI. To account for the potential effects of the scanning environment, baseline cortisol level was defined using an in-scanner draw, conducted approximately 5 min after the subject was introduced into the magnet (after the first few set-up scans) and just before the start of the first stress functional scan (time 0). Two in-scanner blood samples were drawn: between the second and the third functional scan of the stress paradigm (time 15 min) and at time 30 min after task presentations (timed for pituitary responses). Two out-of-scanner blood samples were drawn in a quiet room (times 60 min and 90 min) to assess steroid hormone responses to stress. Subjects remained inside the bore of the magnet during in-scanner blood draws. Table S2 in Supplement 1 provides further details and a complete list of absolute cortisol values. Given significant variability in baseline (prestress) cortisol levels, individuals' cortisol response to stress was calculated as percentage of change from time 0 (i.e., controlling for baseline level in scanner).

fMRI Data Analysis

See Supplement 1 for magnetic resonance imaging data acquisition parameters. The fMRI data were preprocessed using statistical parametric mapping (SPM8; Wellcome Department of Cognitive Neurology, London, United Kingdom) and included realignment and geometric unwarping of echo-planar imaging images using magnetic field maps, correction for head motion, nonlinear volume-based spatial normalization (Montreal Neurological Institute template MNI-152), and spatial smoothing with a Gaussian filter (6 mm [full width at half maximum]). Additional software (http://web.mit.edu/swg/software.htm) was used to identify and exclude outliers in the global mean image time series (threshold 3.5 SD from the mean) and movement (threshold .7 mm; measured as scan-to-scan movement, separately for translation and rotation) parameters.

Hemodynamic responses were modeled using a gamma function and convolved with onset times of negative, neutral, and fixation blocks to form the general linear model at the single subject level. Outlier time points and the six rigid-body movement parameters were included in the general linear model as covariates of no interest. To test a priori hypotheses targeting striatal activations during stress, we conducted region of interest (ROI) analyses in which activations (beta weights) were extracted from anatomic masks of the caudate, Nacc, and putamen for each participant separately for negative and neutral conditions relative to baseline. Anatomical masks for the ROI were defined using a manually segmented MNI-152 brain and implemented as overlays on the SPM8 canonical brain (Figure 2A). For each participant and ROI, activations from the left and right mask were entered into a mixed analysis of variance (ANOVA) with group (control vs. rMDD) and gender (male vs. female) as between-subject factors, side (left vs. right) and condition (negative vs. neutral) as repeated measures, and with or without age and SES as covariates.

Given the lack of laterality effects on activations in any ROI, the left and right masks of each ROI were merged to create a single bilateral mask, from which time courses were extracted for PPI analyses. For each participant, subject-level general linear models were constructed as described earlier, with the addition of the bilateral seed time course as a regressor and two additional PPI regressors (the interaction of the seed time course with the regressors for negative and neutral condition). These interaction regressors are orthogonal to the task and seed regressors and describe the contribution of the interaction above and beyond the main effects of the task and seed time course. In addition, the orthogonality of the task and PPI regressors ensures that seed ROI activation and PPI connectivity are independent (52).

Striatal connectivity was measured at the single subject level by estimating the difference between the interaction of the seed time course with the regressor for negative versus neutral pictures (each relative to baseline), and this was done separately for each ROI. Single subject activation maps were entered into second-level random effects analysis to probe group differences in striatal connectivity during negative versus neutral condition. Given extensive prior evidence for hyperactive amygdala and hippocampus in response to negative stimuli and stress in rMDD (44–49), striatal connectivity was investigated by applying small volume correction on anatomic masks of the amygdala and hippocampus. False-positive findings were controlled using family-wise error (FWE) correction. Average connectivity (beta weights of PPI regressors) in amygdala and hippocampal clusters that survived FWE correction was extracted and entered into a mixed ANOVA with group (control vs. rMDD) and gender (male vs. female) as between-subject factors, condition (negative vs. neutral) as the repeated measure, and with or without age and SES as covariates. Finally, exploratory whole-brain analyses probed group differences in activation and connectivity outside a priori ROIs, with age and SES as covariates, and at uncorrected $p < .005$ in >10 contiguous voxels.

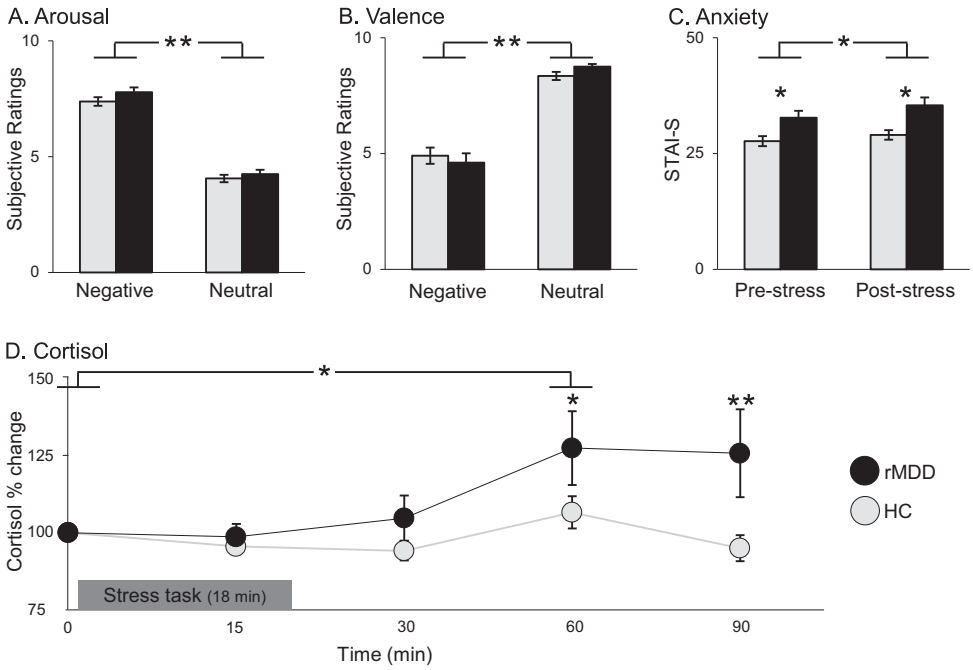

Figure 1. Stress response assessment. Both groups rated the negative stimuli as **(A)** more arousing and **(B)** more negative compared with the neutral stimuli, and both experienced an increase in **(C)** anxiety and **(D)** cortisol levels (60 min after stress onset relative to baseline) following the mild stress paradigm. Relative to healthy control subjects, remitted individuals with a history of recurrent major depressive disorder exhibited overall significantly higher anxiety and cortisol response (60 min and 90 min after stress onset relative to baseline). Bars ± 1 SEM. $^{*}p < .05$, $^{**}p < .001$. HC, healthy control group; rMDD, recurrent major depressive disorder group; STAI-S, Spielberger State-Trait Anxiety Inventory.

RESULTS

Stress Response Assessment

The ANOVA on subjective ratings of neutral and negative stimuli revealed the expected main effect of condition on arousal [$F_{1,63} = 300.42$, $p < .001$] and valence [$F_{1,63} = 185.46$, $p < .001$] scales, with no group by condition interaction (arousal, $p = .242$; valence, $p = .579$). The ANOVA of anxiety before and after scanning (Spielberger State-Trait Anxiety Inventory) revealed a significant main effect of time [$F_{1,57} = 8.11$, $p = .006$] and Group [$F_{1,57} = 9.23$, $p = .004$] but no group by time interaction ($p = .92$). These results indicate that participants across groups rated the negative stimuli as more arousing and more negative compared with the neutral stimuli and experienced increased anxiety after the task; however, rMDD individuals exhibited overall significantly higher anxiety than control subjects (Figure 1A–C).

The ANOVA of cortisol response across the five time points revealed a main effect of Time [$F_{3,168} = 2.72$, $p = .047$], owing to an overall increase in cortisol levels 60 min after the onset of the stress task compared with baseline ($p = .012$). Critically, a significant Group by Time interaction emerged [$F_{3,168} = 3.63$, $p = .014$], due to higher cortisol response in rMDD individuals compared with control subjects both 60 min ($p = .006$) and 90 min ($p < .001$) after stress onset (Figure 1D). Similarly, classifying participants according to whether they demonstrated an increase (responders) or decrease (nonresponders) in cortisol levels from baseline to the expected peak response time (60 min after stress onset) resulted in 69% (22 of 32 with full data) of rMDD individuals being classified as responders compared with 27% (8 of 29 with full data) of control subjects classified as responders. This classification was significantly different between groups ($\chi^2 = 10.3$; $p = .001$), indicating greater cortisol responsivity to stress in rMDD individuals compared with controls. Accordingly, cortisol data confirmed that the task elicited a stress response across participants and that rMDD individuals experienced a more marked response to this mild stressor.

Striatal Activation During Stress

The ANOVAs comparing group activations for negative and neutral conditions separately for each striatal ROI yielded no main effect of group or gender. In the caudate and putamen, there was a significant main effect of condition attributed to overall increased striatal response to negative compared with neutral stimuli (caudate, [$F_{1,60} = 10.63$, $p = .002$]; putamen, [$F_{1,60} = 21.19$, $p < .001$]). Relevant to the study hypotheses, a significant group by condition interaction emerged for all three ROIs (caudate, [$F_{1,60} = 9.84$, $p = .003$]; Nacc, [$F_{1,60} = 4.51$, $p = .038$]; putamen, [$F_{1,60} = 7.58$, $p = .008$]), owing to the fact that the increase in striatal response to negative compared with neutral stimuli was present in the rMDD group but not the control group (caudate, $p < .001$; Nacc, $p = .013$; putamen, $p < .001$) (Figure 2B–D). For the caudate, there was also a significant group difference such that rMDD individuals exhibited increased caudate activation compared with control subjects in response to negative, but not neutral, stimuli ($p = .03$). Analogous analyses accounting for age and SES as covariates confirmed the significant group by condition interactions (caudate, $p = .006$; Nacc, $p = .042$; putamen, $p = .019$).

Finally, regression analyses highlighted a significant positive correlation between caudate activation in response to negative stimuli and peak cortisol release (60 min after stress onset relative to in-scanner baseline) in the rMDD group ($r = .412$, $p = .036$), but not the control group ($r = -.058$, $p = .765$) (Figure 3), and these independent correlations were significantly different ($Z = 1.952$, $p = .05$). Thus, in parallel with their elevated

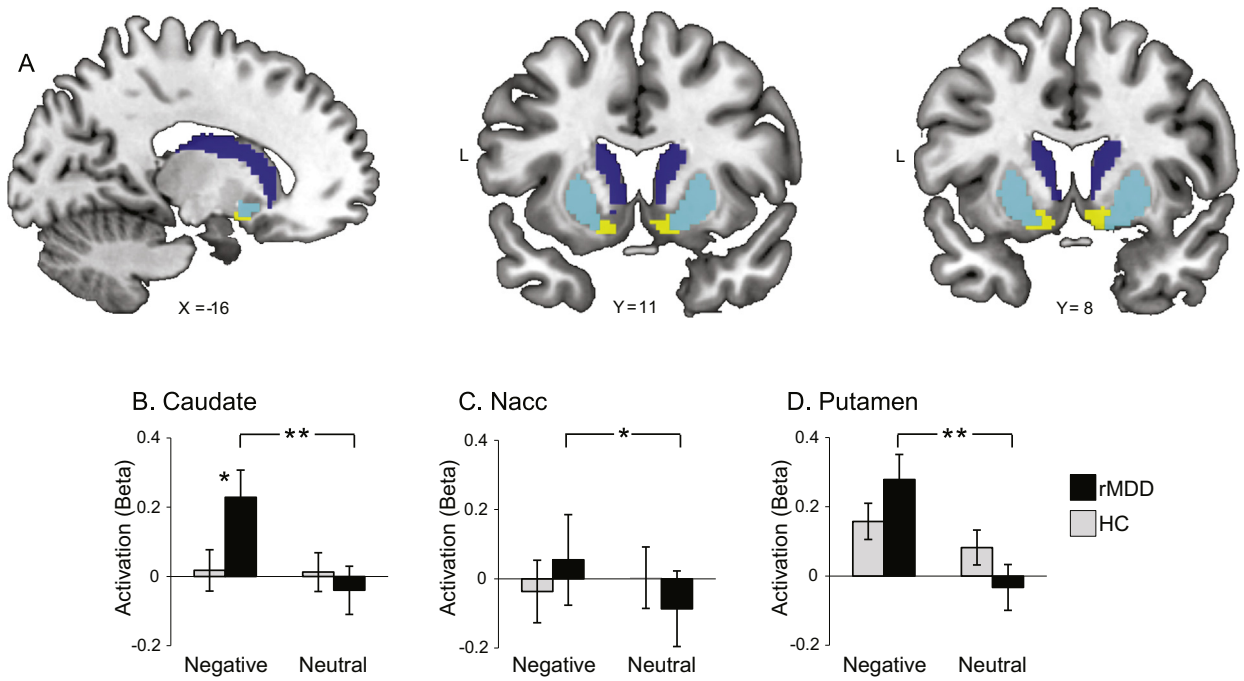

Figure 2. Striatal activation during stress. **(A)** Location of anatomically defined masks for the caudate (blue), nucleus accumbens (yellow), and putamen (turquoise). Mask volumes were 169 voxels and 195 voxels for the left and right caudate, 25 voxels and 34 voxels for the left and right nucleus accumbens, and 226 voxels and 239 voxels for the left and right putamen. Remitted individuals with a history of recurrent major depressive disorder, but not healthy control subjects, exhibited significantly increased activation in all three striatal regions, the **(B)** caudate, **(C)** nucleus accumbens, and **(D)** putamen, in response to the negative stimuli compared with the neutral stimuli. Bars ± 1 SEM. *$p < .05$, **$p < .001$. HC, healthy control group; rMDD, recurrent major depressive disorder group; Nacc, nucleus accumbens.

behavioral and hormonal responses to the mild stress challenge, rMDD individuals, but not control subjects, exhibited significantly increased activations in all three striatal regions in response to the negative stimuli, and such increased activation in the caudate correlated with stress-induced cortisol release.

Striatal Connectivity During Stress

The PPI analyses were focused on group differences in striatal connectivity with the amygdala and hippocampus in response to negative versus neutral stimuli. These analyses revealed two clusters, one in left amygdala and one in left hippocampus, which showed greater functional connectivity with the caudate in rMDD individuals compared with control subjects ($p_{FWE\text{-}corrected} < .05$) (Figure 4A and Table 2). To investigate these results further, connectivity values for each condition (negative vs. neutral) were entered into an ANOVA with group (control vs. rMDD) and gender (male vs. female) as between-subject factors. Main effects of condition, group, and gender were not significant; however, a significant group by condition interaction emerged for both caudate-amygdala and caudate-hippocampus connectivity [$F_{1,63} = 10.92$, $p = .002$; $F_{1,63} = 9.18$, $p = .004$]; or $p = .003$ and $p = .023$ when controlling for Age and SES). Mirroring the activation results, these interactions stemmed from the fact that rMDD individuals, but not control subjects, exhibited increased caudate-amygdala ($p = .013$) and caudate-hippocampus ($p = .009$) connectivity in response to the negative versus neutral stimuli (Figure 4B,C). In addition, for caudate-amygdala connectivity, there was a significant group difference such that rMDD individuals exhibited increased connectivity compared with control subjects in response to the negative, but not the neutral, stimuli ($p = .033$). Similar analyses conducted with

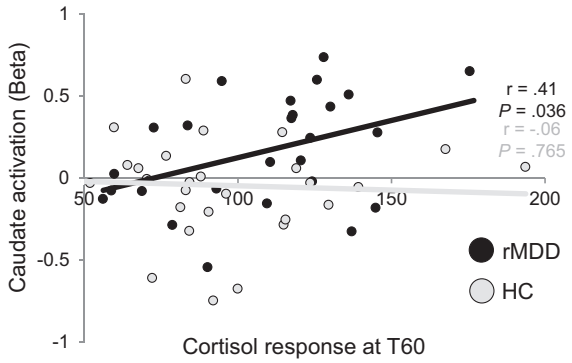

Figure 3. Caudate activation during stress and cortisol release. For remitted individuals with a history of recurrent major depressive disorder ($r = .412$, $p = .036$), but not healthy control subjects ($r = -.058$, $p = .77$), caudate activation in response to the negative stimuli was positively associated with peak cortisol release (60 min after stress onset relative to baseline). Cortisol level at 90 min after stress onset relative to baseline and area under the curve of cortisol release were not correlated with caudate activation in remitted individuals with a history of recurrent major depressive disorder ($r = -.13$, $p = .54$; $r = .05$, $p = .79$) or healthy control subjects ($r = .21$, $p = .38$; $r = -.12$, $p = .60$). HC, healthy control group; rMDD, recurrent major depressive disorder group.

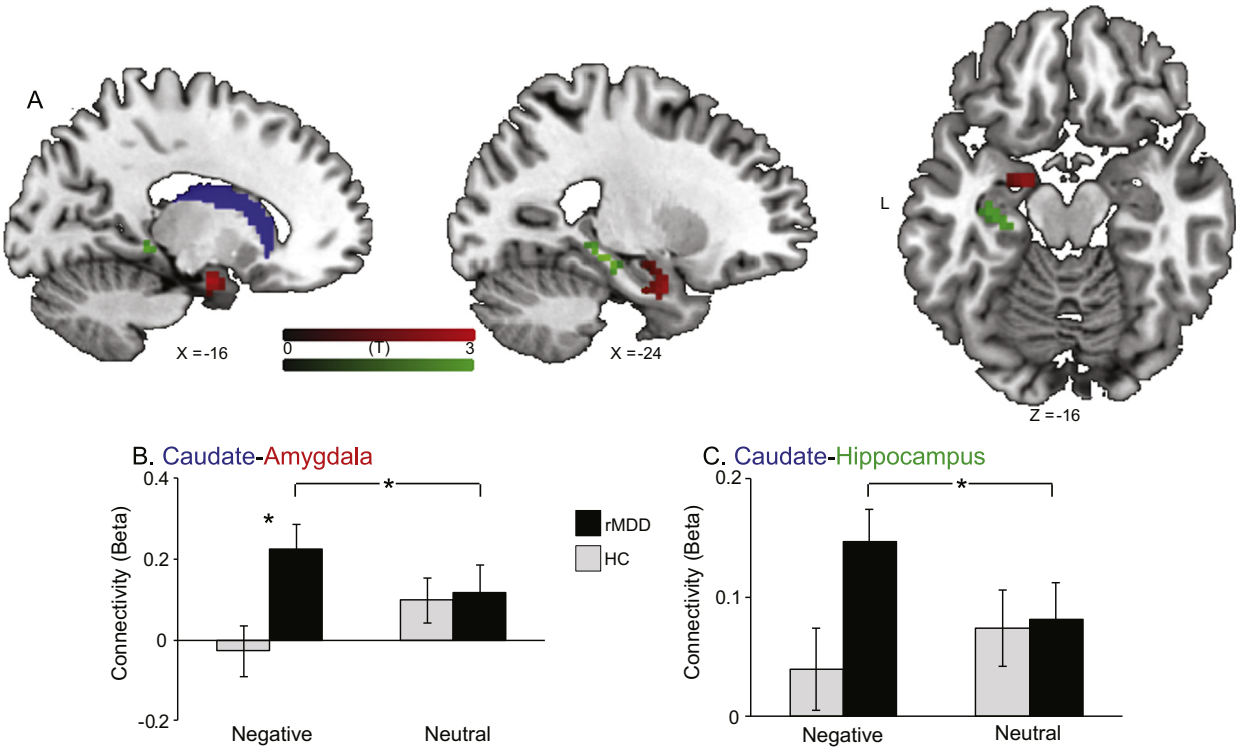

Figure 4. Striatal connectivity during stress. **(A)** Psychophysiologic interaction analyses revealed one cluster in the left amygdala (red) and one in the left hippocampus (green) that were more functionally connected to the caudate (blue) in remitted individuals with a history of recurrent major depressive disorder compared with healthy control subjects during stress ($p < .05$, corrected for family-wise error). Specifically, remitted individuals with a history of recurrent major depressive disorder, but not healthy control subjects, exhibited increased **(B)** caudate-amygdala and **(C)** caudate-hippocampus connectivity in response to the negative stimuli compared with the neutral stimuli. Bars ± 1 SEM. *$p < .05$. HC, healthy control group; rMDD, recurrent major depressive disorder group.

respect to Nacc and putamen connectivity also revealed greater functional connectivity with clusters in left amygdala and left hippocampus in rMDD individuals compared with control subjects during stress; however, none survived FWE correction (Table 2). Finally, even at a liberal threshold of uncorrected $p < .05$, no clusters in the amygdala or hippocampus showed stronger striatal connectivity in control subjects compared with rMDD individuals.

Table 2. Striatal Connectivity Abnormalities During Stress in Remitted Individuals with a History of rMDD

Contrast	Region	No. of Voxels	X[a]	Y[a]	Z[a]	*Z* Score	*T* Value	Uncorrected *P* Value[b]	FWE-Corrected *P* Value[c]
Negative vs. Neutral Stimuli (rMDD Group > HC Group)					Caudate Connectivity				
	L amygdala	60	−15	−7	−20	3.24	3.39	.001	.022[d]
	L hippocampus	22	−33	−19	−14	3.34	3.51	.000	.038[d]
					Nacc Connectivity				
	L amygdala	18	−27	−13	−14	2.43	2.49	.008	.162
	L hippocampus	20	−30	−16	−14	2.59	2.67	.005	.263
					Putamen Connectivity				
	L amygdala	9	−24	−7	−26	2.69	2.77	.004	.098
	L hippocampus	33	−24	−28	−11	3.01	3.13	.001	.108

FWE, family-wise error; HC, healthy control; L, left; Nacc, nucleus accumbens; rMDD, recurrent major depressive disorder.

[a]Coordinates are presented in Montreal Neurological Institute space.

[b]Within an anatomic region of interest, results identified using small volume correction with voxel-wise peak-level height threshold: $p < .05$, uncorrected for multiple comparisons.

[c]False-positive findings controlled using FWE correction. Even at a liberal threshold of uncorrected $p < .05$, no clusters in the amygdala or hippocampus showed stronger striatal connectivity in the healthy control group compared with the rMDD group during stress.

[d]Results are reported as significant only if they met the peak-level threshold of FWE-corrected $p < .05$.

Exploratory Whole-Brain Analyses

In addition to the striatum, bilateral hippocampus activation was also increased in rMDD individuals relative to control subjects in response to negative stimuli. No brain regions were more active in control subjects than rMDD individuals in response to negative stimuli. Whole-brain analyses examining caudate connectivity in response to negative stimuli in rMDD individuals relative to control subjects revealed, as expected, a large cluster encompassing the left amygdala and hippocampus and a cluster in the left fusiform gyrus; see Supplement 1 (Figure S1A, B and Table S1 in Supplement 1). No regions were more connected to the caudate in control subjects than rMDD individuals in response to negative stimuli.

Control Analyses

Correlational analyses revealed no associations between HAM-D_{17} scores among the rMDD group and striatal activation or connectivity magnitudes (all $p > .22$), indicating that the current findings were not modulated by residual depressive symptoms.

DISCUSSION

The goal of the present study was to probe striatal activation and connectivity during mild stress in healthy and remitted individuals with recurrent depression. Affective and endocrinologic findings confirmed a mild psychological stress response, as intended, in both the healthy and the remitted group; however, rMDD individuals exhibited a stronger stress response than controls. The fMRI results indicated that heightened stress responsivity in rMDD individuals was accompanied by increased activation to negative stimuli in three key striatal regions, the caudate, Nacc, and putamen; a pattern that was not found in control subjects. PPI analyses further revealed increased caudate connectivity with both the amygdala and the hippocampus in response to the negative stimuli in rMDD individuals, but not control subjects. Collectively, the findings suggest that rMDD is characterized by striatal hypersensitivity during a mild stressor.

Group differences in cortisol response between rMDD individuals and healthy control subjects fit with previous reports that hypothalamic-pituitary-adrenal axis dysregulation persists after remission from depression. Interestingly, while basal cortisol response seems to be consistently increased after remission from depression (61), stress reactivity studies commonly report reduced cortisol responses in rMDD individuals relative to control subjects, potentially as a result of adaptation to previous stress exposure (62–64), but see also (65). Notably, those studies implemented a relatively potent stressor [e.g., the Trier Social Stress Test (66)], known to elicit robust increases in cortisol levels in healthy humans, which are considered adaptive (67,68). The demonstrated increase in cortisol release in response to mild stress in rMDD individuals relative to control subjects may therefore suggest that remitted individuals are particularly susceptible to the effects of minor stressors; such "hypersensitive" physiologic response to a mild stress challenge is consistent with the kindling/sensitization theory of depression (2,3). Indeed, enhanced cortisol reactivity in rMDD individuals in response to a minor laboratory stressor, but not to a more potent stressor, was found to predict depression relapse in a longitudinal study (69).

Animal work has shown that stress-induced corticosteroid release can modulate DA striatal signaling (70–72). Similarly, human positron emission tomography studies revealed a positive association between cortisol increase and DA release in the ventral striatum and putamen (73,74). Although fMRI cannot be used to infer DA signaling, pharmacologic and metabolic evidence suggests that fMRI blood oxygen level–dependent signal from the striatum is indicative of striatal DA release (53,75–77). Our results thus may be regarded as supportive of a potential relationship between cortisol and striatal function by showing that elevated cortisol release in the rMDD group was accompanied by increased striatal activations and connectivity during stress and furthermore that caudate reactivity and stress-induced cortisol release were positively correlated in the rMDD sample. Along similar lines, positron emission tomography studies found stress-induced striatal DA release in individuals with low parental care (73) or at risk for affective (78,79) or mood disorders (80), but not in healthy control subjects (81,82), again consistent with our results. Findings from animal work suggest that stress-induced striatal DA release may amplify the incentive salience of stimuli (19). In humans, such enhanced saliency may translate to increased attention or emotional engagement during presentation of negative cues, processes that were shown to involve the striatum as well as the amygdala and hippocampus. Over time, striatal hypersensitivity in rMDD, through its association with chronic exposure to high glucocorticoid levels, may sensitize the mesolimbic DA system (76), resulting in increased susceptibility to mild stressors.

Striatal hypersensitivity during stress may be particularly detrimental for the encoding of subsequent rewards, given shared reliance of stress and reward responses on striatal DA signaling (18,19). In support of this possibility, reductions in hedonic behavior after various forms of stress have been reported in the animal (7–11) and human (12–16) literature. Moreover, individuals with heightened cortisol response to stress were found to be specifically vulnerable to the disruptive effect of an acute stressor on reward sensitivity (83). Finally, recent fMRI studies demonstrated reduced reward-related striatal activation in healthy individuals after acute laboratory stress (84,85) and after prolonged combat stress (86), with the latter also linking reward-related striatal blunting with greater severity of depressive symptoms (86). Altogether, when seen in the context of emerging evidence, the current findings suggest that striatal hypersensitivity during stress may play a crucial role in the interaction between increased sensitivity to mild stressors and dysfunctional reward processing, two primary characteristics of MDD that persist after remission.

Additional studies are needed to validate this novel idea and address limitations of the present work. First, throughout this article, we refer to the striatum in general since all three striatal regions (caudate, Nacc, and putamen) exhibited similar activation patterns. Increased connectivity with the amygdala and hippocampus was also consistent across striatal nuclei; although group differences were significant only for the caudate. Nevertheless, work in animals and humans has demonstrated that the function and connections of different striatal nuclei and their subcomponents vary greatly and

include many signaling pathways (87,88). Future studies should delineate the specific involvement of each striatal nuclei in response to stress in both healthy control and rMDD samples. Second, some of the rMDD participants were taking psychotropic medication and presented with current or past comorbidities (or both). Importantly, however, with the exception of Nacc activation, all group differences in activation and connectivity during stress remained significant when analyses were repeated with only rMDD individuals who were not taking psychotropic medication ($n = 20$) or without comorbid diagnoses ($n = 15$), suggesting that medication and comorbidities did not affect our main findings; for more details, see Figures S2 and S3 in Supplement 1. Nevertheless, Nacc results should be regarded with caution given the insignificant group differences in Nacc activation in these subsamples and the observation that group differences in Nacc activation at the whole-sample level may have been partially driven by reduced activation in response to the neutral condition in rMDD. Finally, our study did not include a stress-free control group or a measure of reward sensitivity, and thus we cannot test the specificity of our findings or the effects of stress on reward function. By combining stress and reward manipulations in a single design as well as a stress-free control group, future studies could test whether rMDD individuals require a lower stressor to observe perturbation in reward processing. It would also be useful to compare reward function and stress sensitivity directly among remitted individuals with a history of MDD and individuals with current MDD. For example, a recent study found that both adolescent daughters with current depression of mothers with a history of MDD and daughters with no history of depression of mothers with a history of MDD exhibit reduced striatal response to reward compared with control daughters with no maternal history of psychopathology (89), strengthening the claim that reward dysfunction represents a promising endophenotype of depression. Future studies directly comparing individuals with current MMD and remitted individuals with a history MDD are needed to evaluate whether similar patterns are evident in adult cohorts.

In conclusion, compared with healthy control subjects, remitted individuals with a history of recurrent depression exhibited potentiated cortisol responses and striatal hypersensitivity in response to a mild psychological stress, as manifested by hyperactivation and hyperconnectivity with the amygdala and hippocampus. Striatal hypersensitivity during stress might constitute a trait mark of MDD, providing a potential neural substrate for the interaction between stress and reward dysfunction in depression.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the National Institutes of Health Office for Research on Women's Health and Mental Health Grant No. P50 MH082679 (JMG); Harvard Catalyst | The Harvard Clinical and Translational Science Center Grant No. UL1 RR025758; National Institute of Mental Health Grant No. K01 MH091222 (LMH); National Institute of Mental Health Grant Nos. R01 MH068376 and R01 MH095809 (DAP); and The Adam Corneel Young Investigator Award (McLean Hospital) and a Brain & Behavior Research Foundation Young Investigator Award (RA).

We thank Dr. Daniel D. Dillon and Dr. Michael T. Treadway for providing fruitful comments on previous versions of this article. We thank Jenn Walch, M.Ed., for help with project management and Jo-Ann Donatelli, Ph.D., for her contributions to diagnostic review with Dr. Goldstein.

Over the past 3 years, DAP has received honoraria or consulting fees from Advanced Neuro Technology North America, AstraZeneca, Pfizer, and Servier for activities unrelated to this project. All other authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Center for Depression, Anxiety and Stress Research (RA, DAP), McLean Hospital, Belmont, Massachusetts; McLean Imaging Center (DAP), McLean Hospital, Belmont, Massachusetts; Department of Psychiatry (RA, LMH, JMG, DAP), Harvard Medical School, Cambridge, Massachusetts; Connors Center for Women's Health and Gender Biology (LMH, HA, AR, JMG), Division of Women's Health, Department of Medicine; Department of Psychiatry (LMH, HA, JMG), Brigham & Women's Hospital, Boston, Massachusetts; Athinoula A. Martinos Center (SW-G, JMG), Massachusetts General Hospital and Massachusetts Institute of Technology, Charlestown, Massachusetts; and Department of Brain and Cognitive Sciences (SW-G), Massachusetts Institute of Technology, Cambridge, Massachusetts.

JMG and DAP are joint senior authors.

Address correspondence to Diego A. Pizzagalli, Ph.D., Center for Depression, Anxiety and Stress Research, Room 233C, McLean Hospital, 115 Mill Street, Belmont, MA 02478; E-mail: dap@mclean.harvard.edu.

Received Jun 26, 2014; revised Aug 29, 2014; accepted Sep 19, 2014.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2014.09.019.

REFERENCES

1. Mueller TI, Leon AC, Keller MB, Solomon DA, Endicott J, Coryell W, *et al*. (1999): Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. Am J Psychiatry 156: 1000–1006.
2. Monroe SM, Harkness KL (2005): Life stress, the "kindling" hypothesis, and the recurrence of depression: Considerations from a life stress perspective. Psychol Rev 112:417–445.
3. Post RM (1992): Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. Am J Psychiatry 149:999–1010.
4. Lethbridge R, Allen NB (2008): Mood induced cognitive and emotional reactivity, life stress, and the prediction of depressive relapse. Behav Res Ther 46:1142–1150.
5. Dobson KS, Shaw BF (1987): Specificity and stability of self-referent encoding in clinical depression. J Abnorm Psychol 96:34–40.
6. Pechtel P, Dutra SJ, Goetz EL, Pizzagalli DA (2013): Blunted reward responsiveness in remitted depression. J Psychiatr Res 47: 1864–1869.
7. Rygula R, Abumaria N, Flugge G, Fuchs E, Ruther E, Havemann-Reinecke U (2005): Anhedonia and motivational deficits in rats: Impact of chronic social stress. Behav Brain Res 162:127–134.
8. Von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den Bos R, Spruijt BM (2000): Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. Behav Brain Res 117:137–146.
9. Elizalde N, Gil-Bea FJ, Ramirez MJ, Aisa B, Lasheras B, Del Rio J, *et al*. (2008): Long-lasting behavioral effects and recognition memory

deficit induced by chronic mild stress in mice: effect of antidepressant treatment. Psychopharmacology (Berl) 199:1–14.
10. Willner P, Muscat R, Papp M (1992): Chronic mild stress-induced anhedonia: A realistic animal model of depression. Neurosci Biobehav Rev 16:525–534.
11. Katz RJ (1982): Animal model of depression: Pharmacological sensitivity of a hedonic deficit. Pharmacol Biochem Behav 16:965–968.
12. Bogdan R, Pizzagalli DA (2006): Acute stress reduces reward responsiveness: Implications for depression. Biol Psychiatry 60: 1147–1154.
13. Nikolova Y, Bogdan R, Pizzagalli DA (2012): Perception of a naturalistic stressor interacts with 5-HTTLPR/rs25531 genotype and gender to impact reward responsiveness. Neuropsychobiology 65:45–54.
14. Bogdan R, Santesso DL, Fagerness J, Perlis RH, Pizzagalli DA (2011): Corticotropin-releasing hormone receptor type 1 (CRHR1) genetic variation and stress interact to influence reward learning. J Neurosci 31:13246–13254.
15. Lapate RC, van Reekum CM, Schaefer SM, Greischar LL, Norris CJ, Bachhuber DR, *et al*. (2014): Prolonged marital stress is associated with short-lived responses to positive stimuli. Psychophysiology 51: 499–509.
16. Berenbaum H, Connelly J (1993): The effect of stress on hedonic capacity. J Abnorm Psychol 102:474–481.
17. Pizzagalli DA (2014): Depression, stress, and anhedonia: Toward a synthesis and integrated model. Annu Rev Clin Psychol 10:393–423.
18. Cabib S, Puglisi-Allegra S (2012): The mesoaccumbens dopamine in coping with stress. Neurosci Biobehav Rev 36:79–89.
19. Sesack SR, Grace AA (2010): Cortico-basal ganglia reward network: Microcircuitry. Neuropsychopharmacology 35:27–47.
20. Schultz W (1998): Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27.
21. Bayer HM, Glimcher PW (2005): Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron 47:129–141.
22. Waelti P, Dickinson A, Schultz W (2001): Dopamine responses comply with basic assumptions of formal learning theory. Nature 412:43–48.
23. Tidey JW, Miczek KA (1996): Social defeat stress selectively alters mesocorticolimbic dopamine release: An in vivo microdialysis study. Brain Res 721:140–149.
24. Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ (1989): Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem 52: 1655–1658.
25. Imperato A, Angelucci L, Casolini P, Zocchi A, Puglisi-Allegra S (1992): Repeated stressful experiences differently affect limbic dopamine release during and following stress. Brain Res 577:194–199.
26. O'Doherty JP (2004): Reward representations and reward-related learning in the human brain: Insights from neuroimaging. Curr Opin Neurobiol 14:769–776.
27. Haber SN, Knutson B (2010): The reward circuit: Linking primate anatomy and human imaging. Neuropsychopharmacology 35:4–26.
28. Forbes EE, Christopher May J, Siegle GJ, Ladouceur CD, Ryan ND, Carter CS, *et al*. (2006): Reward-related decision-making in pediatric major depressive disorder: An fMRI study. J Child Psychol Psychiatry 47:1031–1040.
29. Forbes EE, Hariri AR, Martin SL, Silk JS, Moyles DL, Fisher PM, *et al*. (2009): Altered striatal activation predicting real-world positive affect in adolescent major depressive disorder. Am J Psychiatry 166:64–73.
30. Knutson B, Bhanji JP, Cooney RE, Atlas LY, Gotlib IH (2008): Neural responses to monetary incentives in major depression. Biol Psychiatry 63:686–692.
31. Kumar P, Waiter G, Ahearn T, Milders M, Reid I, Steele JD (2008): Abnormal temporal difference reward-learning signals in major depression. Brain 131:2084–2093.
32. Steele JD, Kumar P, Ebmeier KP (2007): Blunted response to feedback information in depressive illness. Brain 130:2367–2374.
33. Pizzagalli DA, Holmes AJ, Dillon DG, Goetz EL, Birk JL, Bogdan R, *et al*. (2009): Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. Am J Psychiatry 166:702–710.
34. Moses-Kolko EL, Fraser D, Wisner KL, James JA, Saul AT, Fiez JA, *et al*. (2011): Rapid habituation of ventral striatal response to reward receipt in postpartum depression. Biol Psychiatry 70:395–399.
35. Dichter GS, Kozink RV, McClernon FJ, Smoski MJ (2012): Remitted major depression is characterized by reward network hyperactivation during reward anticipation and hypoactivation during reward outcomes. J Affect Disord 136:1126–1134.
36. McCabe C, Cowen PJ, Harmer CJ (2009): Neural representation of reward in recovered depressed patients. Psychopharmacology (Berl) 205:667–677.
37. McEwen BS (2007): Physiology and neurobiology of stress and adaptation: Central role of the brain. Physiol Rev 87:873–904.
38. Phillips RG, LeDoux JE (1992): Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285.
39. Dedovic K, Duchesne A, Andrews J, Engert V, Pruessner JC (2009): The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. Neuroimage 47:864–871.
40. Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA (2001): Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: An fMRI study. Biol Psychiatry 50:651–658.
41. Fahim C, Stip E, Mancini-Marie A, Mensour B, Leroux JM, Beaudoin G, *et al*. (2004): Abnormal prefrontal and anterior cingulate activation in major depressive disorder during episodic memory encoding of sad stimuli. Brain Cogn 54:161–163.
42. Groenewold NA, Opmeer EM, de Jonge P, Aleman A, Costafreda SG (2013): Emotional valence modulates brain functional abnormalities in depression: Evidence from a meta-analysis of fMRI studies. Neurosci Biobehav Rev 37:152–163.
43. Surguladze S, Brammer MJ, Keedwell P, Giampietro V, Young AW, Travis MJ, *et al*. (2005): A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biol Psychiatry 57:201–209.
44. Ramel W, Goldin PR, Eyler LT, Brown GG, Gotlib IH, McQuaid JR (2007): Amygdala reactivity and mood-congruent memory in individuals at risk for depressive relapse. Biol Psychiatry 61:231–239.
45. Pulcu E, Lythe K, Elliott R, Green S, Moll J, Deakin JF, *et al*. (2014): Increased amygdala response to shame in remitted major depressive disorder. PloS One 9:e86900.
46. Hooley JM, Gruber SA, Parker HA, Guillaumot J, Rogowska J, Yurgelun-Todd DA (2009): Cortico-limbic response to personally challenging emotional stimuli after complete recovery from depression. Psychiatry Res 172:83–91.
47. Neumeister A, Drevets WC, Belfer I, Luckenbaugh DA, Henry S, Bonne O, *et al*. (2006): Effects of an alpha 2C-adrenoreceptor gene polymorphism on neural responses to facial expressions in depression. Neuropsychopharmacology 31:1750–1756.
48. Victor TA, Furey ML, Fromm SJ, Ohman A, Drevets WC (2010): Relationship between amygdala responses to masked faces and mood state and treatment in major depressive disorder. Arch Gen Psychiatry 67:1128–1138.
49. Holsen LM, Lancaster K, Klibanski A, Whitfield-Gabrieli S, Cherkerzian S, Buka S, *et al*. (2013): HPA-axis hormone modulation of stress response circuitry activity in women with remitted major depression. Neuroscience 250:733–742.
50. O'Reilly JX, Woolrich MW, Behrens TE, Smith SM, Johansen-Berg H (2012): Tools of the trade: Psychophysiological interactions and functional connectivity. Soc Cogn Affect Neurosci 7:604–609.
51. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997): Psychophysiological and modulatory interactions in neuroimaging. Neuroimage 6:218–229.
52. McLaren DG, Ries ML, Xu G, Johnson SC (2012): A generalized form of context-dependent psychophysiological interactions (gPPI): A comparison to standard approaches. Neuroimage 61:1277–1286.
53. Knutson B, Gibbs SE (2007): Linking nucleus accumbens dopamine and blood oxygenation. Psychopharmacology (Berl) 191:813–822.
54. Niswander KR, Gordon M (1972): The women and their pregnancies: The collaborative perinatal study of the National Institute of

Neurological Diseases and Stroke. U.S. Department of Health, Education, and Welfare. Washington, DC: U.S. Government Printing Office.
55. Hamilton M (1960): A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62.
56. Goldstein JM, Jerram M, Abbs B, Whitfield-Gabrieli S, Makris N (2010): Sex differences in stress response circuitry activation dependent on female hormonal cycle. J Neurosci 30:431–438.
57. Holsen LM, Spaeth SB, Lee JH, Ogden LA, Klibanski A, Whitfield-Gabrieli S, *et al*. (2011): Stress response circuitry hypoactivation related to hormonal dysfunction in women with major depression. J Affect Disord 131:379–387.
58. Goldstein JM, Jerram M, Poldrack R, Ahern T, Kennedy DN, Seidman LJ, *et al*. (2005): Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. J Neurosci 25:9309–9316.
59. Bradley MM, Lang PJ (1994): Measuring emotion: The Self-Assessment Manikin and the Semantic Differential. J Behav Ther Exp Psychiatry 25:49–59.
60. Spielberger CD, Gorsuch RL, Lushene R, Vagg IR, Jacobs GA (1983): Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologist Press.
61. Cowen PJ (2010): Not fade away: The HPA axis and depression. Psychol Med 40:1–4.
62. Morris MC, Rao U, Wang L, Garber J (2014): Cortisol reactivity to experimentally manipulated psychosocial stress in young adults at varied risk for depression. Depress Anxiety 31:44–52.
63. Ahrens T, Deuschle M, Krumm B, van der Pompe G, den Boer JA, Lederbogen F (2008): Pituitary-adrenal and sympathetic nervous system responses to stress in women remitted from recurrent major depression. Psychosom Med 70:461–467.
64. Bagley SL, Weaver TL, Buchanan TW (2011): Sex differences in physiological and affective responses to stress in remitted depression. Physiol Behav 104:180–186.
65. Lange C, Zschucke E, Ising M, Uhr M, Bermpohl F, Adli M (2013): Evidence for a normal HPA axis response to psychosocial stress in patients remitted from depression. Psychoneuroendocrinology 38: 2729–2736.
66. Kirschbaum C, Pirke KM, Hellhammer DH (1993): The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28:76–81.
67. Dickerson SS, Kemeny ME (2004): Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. Psychol Bull 130:355–391.
68. de Kloet ER, Oitzl MS, Joels M (1999): Stress and cognition: Are corticosteroids good or bad guys? Trends Neurosci 22:422–426.
69. Morris MC, Rao U, Garber J (2012): Cortisol responses to psychosocial stress predict depression trajectories: Social-evaluative threat and prior depressive episodes as moderators. J Affect Disord 143:223–230.
70. Barrot M, Marinelli M, Abrous DN, Rouge-Pont F, Le Moal M, Piazza PV (2000): The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. Eur J Neurosci 12:973–979.
71. Cho K, Little HJ (1999): Effects of corticosterone on excitatory amino acid responses in dopamine-sensitive neurons in the ventral tegmental area. Neuroscience 88:837–845.
72. Tye SJ, Miller AD, Blaha CD (2009): Differential corticosteroid receptor regulation of mesoaccumbens dopamine efflux during the peak and nadir of the circadian rhythm: A molecular equilibrium in the midbrain? Synapse 63:982–990.
73. Pruessner JC, Champagne F, Meaney MJ, Dagher A (2004): Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: A positron emission tomography study using [11C]raclopride. J Neurosci 24:2825–2831.
74. Oswald LM, Wong DF, McCaul M, Zhou Y, Kuwabara H, Choi L, *et al*. (2005): Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. Neuropsychopharmacology 30:821–832.
75. Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD (2006): Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. Nature 442:1042–1045.
76. Schott BH, Minuzzi L, Krebs RM, Elmenhorst D, Lang M, Winz OH, *et al*. (2008): Mesolimbic functional magnetic resonance imaging activations during reward anticipation correlate with reward-related ventral striatal dopamine release. J Neurosci 28:14311–14319.
77. Oei NY, Rombouts SA, Soeter RP, van Gerven JM, Both S (2012): Dopamine modulates reward system activity during subconscious processing of sexual stimuli. Neuropsychopharmacology 37:1729–1737.
78. Mizrahi R, Addington J, Rusjan PM, Suridjan I, Ng A, Boileau I, *et al*. (2012): Increased stress-induced dopamine release in psychosis. Biol Psychiatry 71:561–567.
79. Soliman A, O'Driscoll GA, Pruessner J, Holahan AL, Boileau I, Gagnon D, *et al*. (2008): Stress-induced dopamine release in humans at risk of psychosis: A [11C]raclopride PET study. Neuropsychopharmacology 33:2033–2041.
80. Mickey BJ, Sanford BJ, Love TM, Shen PH, Hodgkinson CA, Stohler CS, *et al*. (2012): Striatal dopamine release and genetic variation of the serotonin 2C receptor in humans. J Neurosci 32:9344–9350.
81. Lataster J, Collip D, Ceccarini J, Haas D, Booij L, van Os J, *et al*. (2011): Psychosocial stress is associated with in vivo dopamine release in human ventromedial prefrontal cortex: A positron emission tomography study using [(1)(8)F]fallypride. Neuroimage 58:1081–1089.
82. Nagano-Saito A, Dagher A, Booij L, Gravel P, Welfeld K, Casey KF, *et al*. (2013): Stress-induced dopamine release in human medial prefrontal cortex—18F-fallypride/PET study in healthy volunteers. Synapse 67:821–830.
83. Berghorst LH, Bogdan R, Frank MJ, Pizzagalli DA (2013): Acute stress selectively reduces reward sensitivity. Front Hum Neurosci 7:133.
84. Porcelli AJ, Lewis AH, Delgado MR (2012): Acute stress influences neural circuits of reward processing. Front Neurosci 6:157.
85. Oei NY, Both S, van Heemst D, van der Grond J (2014): Acute stress-induced cortisol elevations mediate reward system activity during subconscious processing of sexual stimuli. Psychoneuroendocrinology 39:111–120.
86. Admon R, Lubin G, Rosenblatt JD, Stern O, Kahn I, Assaf M, *et al*. (2013): Imbalanced neural responsivity to risk and reward indicates stress vulnerability in humans. Cereb Cortex 23:28–35.
87. Cai X, Kim S, Lee D (2011): Heterogeneous coding of temporally discounted values in the dorsal and ventral striatum during intertemporal choice. Neuron 69:170–182.
88. Wickens JR, Budd CS, Hyland BI, Arbuthnott GW (2007): Striatal contributions to reward and decision making: Making sense of regional variations in a reiterated processing matrix. Ann N Y Acad Sci 1104:192–212.
89. Sharp C, Kim S, Herman L, Pane H, Reuter T, Strathearn L (2014): Major depression in mothers predicts reduced ventral striatum activation in adolescent female offspring with and without depression. J Abnorm Psychol 123:298–309.

Striatal Hyper-Sensitivity during Stress in Remitted Individuals with Recurrent Depression

Supplemental Information

Supplementary Methods and Materials

MRI Data Acquisition

MRI scanning was conducted using a Siemens Tim Trio 3T MR scanner with a 12-channel head coil. One hundred eighty functional volumes were acquired using a T2-weighted spin echo planar imaging sequence [repetition time = 2000 ms; echo time = 40 ms; field of view = 200 x 200 mm; matrix = 64 x 64; in-plane resolution = 3.125 mm; slice thickness = 5 mm; 23 contiguous slices aligned to the AC–PC plane].

Whole Brain Analyses Probing Group Differences in Activation and Connectivity during Stress

Single subject activation maps in response to the negative stimuli vs. baseline were entered into second-level random effects analysis to probe group differences in activation at the whole brain level (while masking out striatal regions), with *Age* and *SES* as covariates, and at $p < 0.005$ uncorrected in more than 10 contiguous voxels. Similarly, whole brain second-level random effects analysis were conducted to examine group differences with regard to caudate connectivity in response to negative stimuli vs. baseline, at a significance level of $p < 0.005$ uncorrected in more than 10 contiguous voxels.

Analyses to Control for Medication and Comorbidity in rMDD Sample

Because some of the rMDD participants in the current sample were taking psychotropic medication and presented current and/or past comorbidities, control analyses investigating group

differences in activation and connectivity during stress were conducted solely with rMDD individuals who were not taking psychotropic medication (n = 20) or without comorbid diagnoses (n = 15). Analyses on those sub-samples were conducted identically to the ones described in the main text with the entire sample. Specifically, group differences in striatal activation were probed by entering activations from the left and right mask for each participant and ROI into a mixed ANOVA with *Group* (HC vs. rMDD) and *Gender* (Male vs. Female) as between-subject factors, *Side* (Left vs. Right) and *Condition* (Negative vs. Neutral) as repeated measures, and *Age* and *SES* as covariates. Connectivity was assessed by entering caudate connectivity magnitude with the amygdala and hippocampal into a mixed ANOVA with *Group* (HC vs. rMDD) and *Gender* (Male vs. Female) as between-subject factors, *Condition* (Negative vs. Neutral) as the repeated measure, and *Age* and *SES* as covariates.

Supplementary Results

Whole Brain Analyses Probing Group Differences in Activation and Connectivity during Stress

Two clusters emerged as being more active in rMDD relative to HC in response to negative stimuli, located in the left and right hippocampus (Figure S1A and Table S1). No brain regions were more active in HC than rMDD in response to the negative stimuli. Whole brain connectivity analyses revealed, as expected, a large cluster encompassing the left hippocampus and amygdala which was more connected to the caudate in rMDD than HC, as well as a cluster in the left fusiform gyrus (Figure S1B and Table S1). No brain regions were more connected to the caudate in HC compared to rMDD in response to the negative stimuli.

Striatal Activation and Connectivity during Stress in Unmedicated rMDD Individuals vs. Controls

Repeated measure ANOVAs comparing group activations for negative and neutral conditions separately for each striatal ROI yielded no main effect of *Group* or *Gender*. In the caudate and putamen there was a significant main effect of *Condition* attributed to overall increased striatal responses to negative compared to neutral stimuli (Caudate, $F_{1,54} = 5.89$, $p = 0.019$; Putamen, $F_{1,54} = 19.87$, $p < 0.001$). Similar to the results with the entire rMDD sample, a significant *Group* by *Condition* interaction emerged for the caudate and putamen (Caudate, $F_{1,54} = 5.38$, $p = 0.024$; Putamen, $F_{1,54} = 7.10$, $p = 0.010$), due to the fact that the increase in striatal responses to negative compared to neutral stimuli was present in the rMDD, but not control, group (Caudate, $p < 0.001$; Putamen, $p < 0.001$). The *Group* by *Condition* interaction in the Nacc was not significant ($F_{1,54} = 2.28$, $p = 0.137$). For the caudate, there was also a significant group difference such that rMDD exhibited increased caudate activation compared to controls in response to negative but not neutral stimuli ($p = 0.02$) (Figure S2 A-C).

Repeated measure ANOVAs comparing group connectivity values for negative and neutral conditions revealed a significant *Group* by *Condition* interaction for both caudate-amygdala and caudate-hippocampus connectivity ($F_{1,54} = 6.67$, $p = 0.013$; $F_{1,54} = 7.52$, $p = 0.008$, respectively), due to the fact that rMDD exhibited increased connectivity compared to controls in response to the negative but not neutral stimuli ($p = 0.011$; $p = 0.023$, respectively) (Figure S2D & E).

Striatal Activation and Connectivity during Stress in Non-comorbid rMDD Individuals vs. Controls

Repeated measure ANOVAs comparing group activations for negative and neutral conditions separately for each striatal ROI yielded no main effect of *Group* or *Gender* but a

significant main effect of *Condition* in the caudate and putamen, attributed to overall increased striatal response to negative compared to neutral stimuli (Caudate, $F_{1,49} = 8.63$, $p = 0.005$; Putamen, $F_{1,49} = 22.06$, $p < 0.001$). Similar to the results with the entire rMDD sample and with the unmedicated rMDD individuals, a significant *Group* by *Condition* interaction emerged for the caudate and putamen (Caudate, $F_{1,49} = 8.06$, $p = 0.007$; Putamen, $F_{1,49} = 9.56$, $p = 0.003$), due to the fact that the increase in striatal responses to negative compared to neutral stimuli was present in the rMDD, but not control, group (Caudate, $p = 0.001$; Putamen, $p < 0.001$). The *Group* by *Condition* interaction in the Nacc was not significant ($F_{1,49} = 2.07$, $p = 0.157$). For the caudate, there was also a significant group difference such that rMDD exhibited increased caudate activation compared to controls in response to negative but not neutral stimuli ($p = 0.008$) (Figure S3 A-C).

Finally, for both caudate-amygdala and caudate-hippocampus connectivity, repeated measure ANOVAs yielded a significant *Group* by *Condition* interaction even when only non-comorbid rMDD individuals were included ($F_{1,49} = 3.91$, $p = 0.049$; $F_{1,49} = 7.08$, $p = 0.011$, respectively). As before, those results were driven by increased connectivity in rMDD compared to controls in response to the negative but not neutral stimuli ($p = 0.024$; $p = 0.029$, respectively) (Figure S3D & E).

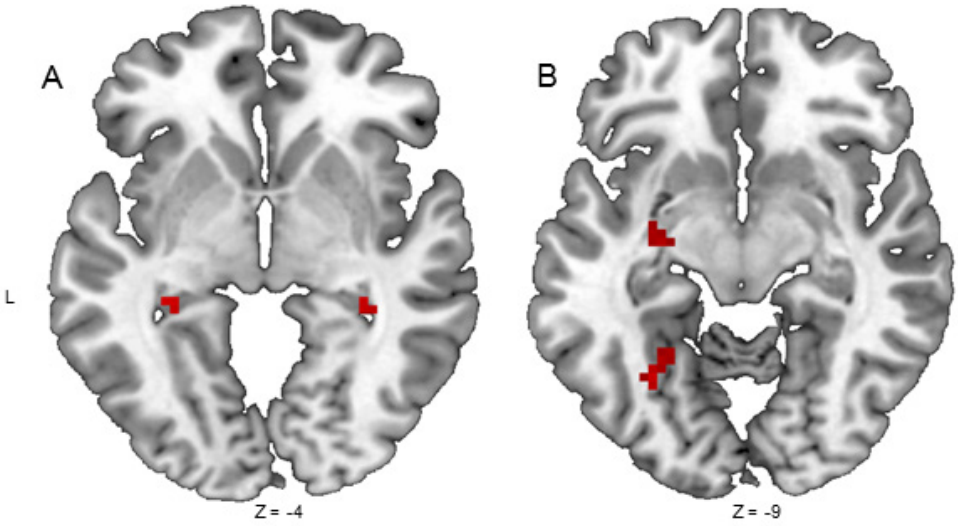

Figure S1. Whole brain analyses probing group differences in activation and connectivity during stress. (A) Two clusters emerged as being more active in rMDD relative to healthy controls in response to the negative stimuli while masking out striatal regions. Those clusters were located in the left and right hippocampus. **(B)** Whole brain psychophysiological interaction connectivity analyses revealed, as expected, a large cluster encompassing the left hippocampus and amygdala which was more connected to the caudate in rMDD than healthy controls in response to the negative stimuli, as well as a cluster in the left fusiform gyrus. No brain regions were more active or more functionally connected to the caudate in healthy controls than rMDD in response to the negative stimuli. rMDD, remitted individuals with a history of recurrent major depressive disorder.

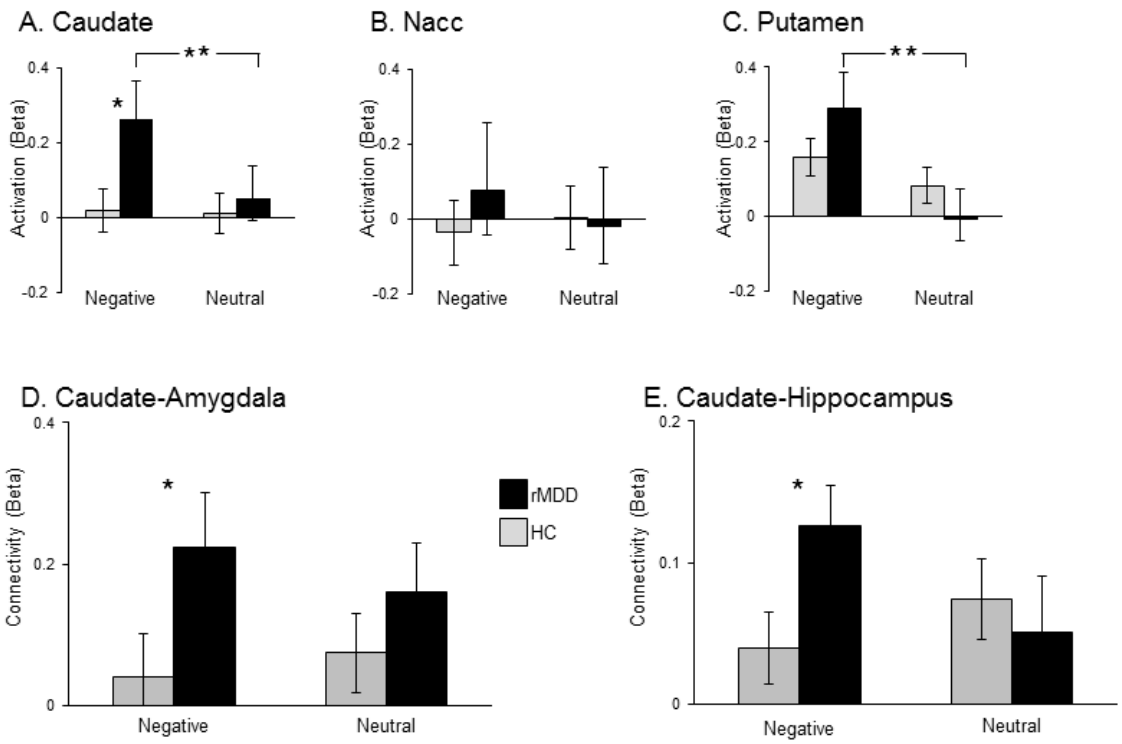

Figure S2. Striatal activation and connectivity during stress in unmedicated rMDD individuals vs. healthy controls. Unmedicated rMDD individuals (n = 20), but not controls (n = 35), exhibited significantly increased activation in the **(A)** caudate and **(C)** putamen, but not the **(B)** Nacc, as well as **(D)** increased caudate-amygdala and **(E)** caudate-hippocampus connectivity in response to the negative stimuli compared to the neutral stimuli. Bars ±1 SEM. *p < 0.05, **p < 0.001. rMDD, remitted individuals with a history of recurrent major depressive disorder; HC, healthy controls; Nacc, nucleus accumbens.

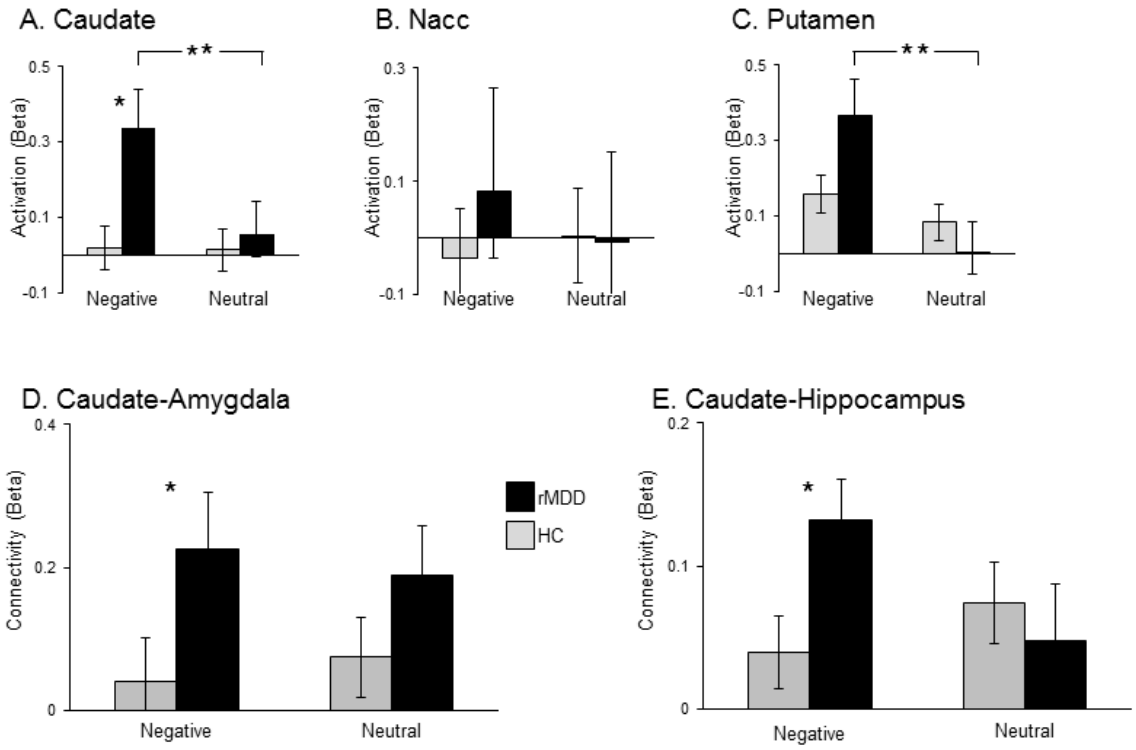

Figure S3. Striatal activation and connectivity during stress in non-comorbid rMDD individuals vs. healthy controls. Non-comorbid rMDD individuals (n = 15), but not controls (n = 35), exhibited significantly increased activation in the **(A)** caudate and **(C)** putamen, but not the **(B)** Nacc, as well as **(D)** increased caudate-amygdala and **(E)** caudate-hippocampus connectivity in response to the negative stimuli compared to the neutral stimuli. Bars ±1 SEM. *p < 0.05, **p < 0.001. rMDD, remitted individuals with a history of recurrent major depressive disorder; HC, healthy controls; Nacc, nucleus accumbens.

Table S1. Whole brain analyses probing group differences in activation and connectivity during stress.

Contrast	Region	# of voxels	X[a]	Y[a]	Z[a]	Z score	T value	Un-corrected *p* value[b]
				Activation				
Negative stimuli vs. Baseline *(rMDD > HC)*	L. Hippocampus	13	-33	-34	-5	2.86	2.97	0.002
	R. Hippocampus	15	33	-34	4	3.07	3.20	0.001
				Caudate Connectivity				
	L. Amygdala/ Hippocampus	55	-30	-7	-11	3.33	3.49	> 0.001
	L. Fusiform gyrus	11	-30	-61	-8	3.47	3.31	> 0.001

[a] Coordinates are presented in MNI space.
[b] Results identified at $p < 0.005$ uncorrected for multiple comparisons, in more than 10 contiguous voxels.
L, left; R, right; rMDD, remitted individuals with a history of recurrent MDD; HC, healthy controls.

Table S2. Absolute cortisol values per subject per time point. Cortisol stress response was assessed using serial blood samples collected during fMRI scanning. Cortisol at T0: In-scanner draw, conducted approximately five minutes after the subject was introduced into the magnet (after the first few set-up scans) and just prior to the start of the first stress functional scan. Cortisol at T15: In-scanner draw, between the second and the third functional scan of the stress paradigm. Cortisol at T30: In-scanner draw, post-task presentations. Subjects remained inside the bore of the magnet during in-scanner blood draws, which were timed to assess pituitary responses. Cortisol at T60 & T90: Out-of-scanner draws, drawn in a quiet room to assess steroid hormone responses to stress. Approximately 30 cc of blood were sampled at each time point, allowed to clot for 45–60 minutes, spun, aliquoted, stored at -80°C, and analyzed in duplicate with a commercial immunoassay kit for cortisol; Immunoradiometric Assay (IRMA), DiaSorin, Inc., Stillwater, MN.

#	Group	Gender	Cortisol at T0	Cortisol at T15	Cortisol at T30	Cortisol at T60	Cortisol at T90
1	HC	M	9.15	7.93	6.54	11.31	9.11
2	HC	M	14.73	12.29	11.73	14.73	7.12
3	HC	M	9.08	8.46	6.54	6.14	13.54
4	HC	M	8.72	7.15	7.54	7.24	11.04
5	HC	M	7.82	6.58	8.47	8.95	8.45
6	HC	M	16.05	15.14	13.36	11.3	9.26
7	HC	M	13.7	14.57	13.56	12.17	8.78
8	HC	M	13.38	12.18	9.7	6.94	5.85
9	HC	M	13.7	14.12	10.39	9.75	10.91
10	HC	M	11.45	16.29	13.99	9.65	7.85
11	HC	M	7.26	6.22	4.08	14.06	9.55
12	HC	M	14.7	15.3	11.3	16.9	13.1
13	HC	M	7.44	7.11	9.28	12.47	10.91
14	HC	M	7.68	8.01	8.18	9.95	7.95
15	HC	M	9.28	8.1	7.98	7.55	10.36
16	HC	M	13.93	12.66	11.88	11.73	11.12
17	HC	M	12.4	10.6	14.4	10.3	11.4
18	HC	F	9.06	8.57	7.9	8.2	8.23
19	HC	F	19.1	18.2	16.6	16.8	13.7
20	HC	F	13.76	11.49	9.88	6.84	5.66
21	HC	F	4.51	5.44	5.3	4.37	3.01
22	HC	F	10	7.36	8.32	6.41	5.67
23	HC	F	5.98	5.83	7.15	8.33	8.18
24	HC	F	10.87	10.61	10.2	10	9.12
25	HC	F	9.27	9.1	8.55	8.92	8.19
26	HC	F	7.09	6.37	6.24	8.19	7.928

#	Group	Gender	Cortisol at T0	Cortisol at T15	Cortisol at T30	Cortisol at T60	Cortisol at T90
27	HC	F	15.33	12.56	12.84	11.06	9.11
28	HC	F	8.36	6.39	5.72	6.41	6.71
29	HC	F	17.1	12.51	12.29	10.21	7.73
30	rMDD	M	13.26	9.978	9.18	9.63	8.66
31	rMDD	M	10.05	9.24	8.16	12.86	6.17
32	rMDD	M	15.55	12.63	11.13	9.73	8.08
33	rMDD	M	11.12	11.99	15.18	14.47	10.75
34	rMDD	M	7.94	5.42	5.33	11.5	7.91
35	rMDD	M	7.44	8.95	7	5.12	4.58
36	rMDD	M	9.13	7.39	6.18	7.64	9.3
37	rMDD	M	5.53	8.41	15.2	16.8	13.52
38	rMDD	M	3.43	2.39	2.3	2.7	6.34
39	rMDD	M	6.33	7.76	9.47	9.4	5.78
40	rMDD	M	6.8	6.78	7.09	8.02	9.48
41	rMDD	M	5.28	4.95	4.58	4.7	4.83
42	rMDD	M	4.51	5.12	4.54	5.94	4.15
43	rMDD	M	3.74	3.48	4.9	15.17	18.49
44	rMDD	M	8.49	14.61	14.43	15.01	10.2
45	rMDD	M	16.17	19.6	17.17	14.58	11.24
46	rMDD	F	10.77	7.74	7.37	13.55	12.75
47	rMDD	F	7.61	6.22	7.42	8.92	9.38
48	rMDD	F	6.01	5.52	7.55	8.24	10.72
49	rMDD	F	8.82	6.33	6.09	10.9	9.85
50	rMDD	F	9.11	10.24	11.96	12.11	12.42
51	rMDD	F	7.54	6.89	6.14	10.26	8.15
52	rMDD	F	5.45	5.36	5.25	5.07	6.69
53	rMDD	F	6.96	9.51	10.62	8.39	10.75
54	rMDD	F	7.66	6.46	6.53	7.26	6.07
55	rMDD	F	5.78	4.75	6.52	6.79	4.15
56	rMDD	F	7.19	5.88	5.55	4.23	16.64
57	rMDD	F	12.4	9.77	15.92	15.4	9.69
58	rMDD	F	8.84	9.41	9.16	9.77	6.38
59	rMDD	F	3.23	3.48	2.98	5.67	5.06
60	rMDD	F	12.23	10.78	9.09	7.31	11.55
61	rMDD	F	11.29	9.44	8.66	16.42	13.53

rMDD, remitted individuals with a history of recurrent major depressive disorder; HC, healthy controls; F, female; M, male.