

# Decreased Hypothalamic Functional Connectivity with Subgenual Cortex in Psychotic Major Depression

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Hypothalamus communication with the rest of the brain and peripheral target tissues is critically important for many physiological and psychological functions. These functions include maintaining neuroendocrine circadian rhythms and managing affective processes. The hypothalamus maintains both direct neural connections within the brain and it also controls a variety of neuroendocrine processes that can influence target tissues throughout the body. Dysregulation of the hypothalamic pituitary adrenal axis and hyperactivity of the subgenual cortex are both frequently observed in depression. However, many details of how the hypothalamus, the hypothalamic pituitary adrenal (HPA) axis, and the subgenual cingulate interact with each other are unknown. We hypothesized that resting-state functional connectivity between the hypothalamus and the subgenual cortex would be associated with altered circadian rhythm in patients with depression and depressive symptoms. We also hypothesized that this would be most apparent in patients that have major depression with psychotic symptoms, who typically have the most robust HPA-axis dysregulation. Resting-state functional magnetic resonance imaging (fMRI) scans were collected to observe low-frequency resting-state functional connectivity patterns of the hypothalamus in 39 healthy participants, 39 patients with major depression, and 22 patients with major depression with psychotic symptoms. Hourly overnight measures of cortisol secretion and multiple measures of psychiatric symptom severity were also collected on all. Strong hypothalamic functional connectivity with the subgenual cortex was observed in healthy participants. This connectivity was significantly reduced in patients with psychotic major depression. Increased cortisol secretion during the circadian nadir and reduced connectivity were both associated with symptom severity. Reduced connectivity and high cortisol secretion during the circadian nadir are both useful for explaining a significant amount of variance in symptom severity that occurs between healthy participants and depressed patients. However, only cortisol secretion was useful for explaining the severity of symptoms within the depressed groups. This study suggests that the communication between the hypothalamus and the subgenual cortex is disrupted in patients with major depression with psychotic features. It also suggests that these disruptions are associated with increased symptom severity and may be a cause or a consequence of cortisol dysregulation.

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## INTRODUCTION

Hypothalamus communication with the rest of the brain is critical for accomplishing a wide variety of physiological and psychological functions, including maintaining neuroendocrine circadian rhythms and managing affective processes. To accomplish these functions, the hypothalamus maintains both neural connections within the brain and also coordinates a variety of neuroendocrine cascades that can influence target tissue throughout the body. Little is known about how these neural and neuroendocrine func-

tions of the hypothalamus interact with each other in healthy individuals or in individuals with psychiatric diseases that are characterized by neuroendocrine dysregulation, such as major depression.

Dysregulation or hyperactivity of the hypothalamic pituitary adrenal (HPA) axis is well characterized in major depression (Hamani *et al*, 2011; Stetler and Miller, 2011). Through the HPA axis, the hypothalamus drives both the acute cortisol response to stress and cortisol secretion in a circadian rhythm. Several types of HPA disturbances are associated with depression, including cortisol hypersecretion (Carroll *et al*, 1976), reduced glucocorticoid receptor mRNA expression (Webster *et al*, 2002), and decreased glucocorticoid-induced inhibitory feedback to the HPA axis (Greden *et al*, 1983; Zobel *et al*, 1999; 2001). Although not all patients with major depression have HPA axis dysregulation, this finding is commonly observed across many studies of depressed patients. The depression subtype that

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shows the most consistent and robust HPA dysregulation in major depression with psychotic features (Stetler and Miller, 2011). These patients present with elevated nocturnal cortisol secretion (Keller *et al.*, 2006) and decreased sensitivity to inhibitory HPA axis feedback mediated by both glucocorticoid receptor (Nelson and Davis, 1997) and mineralocorticoid receptor (Lembke *et al.*, 2012).

Subgenual cortex hyperactivity is another commonly observed phenomenon in patients with depression. The subgenual cortex is highly active during the normal transient experience of sadness in healthy individuals and is hyperactive in depressed patients (Mayberg *et al.*, 1999; Phan *et al.*, 2002). This hyperactivity has been shown to correlate with the severity of depressive symptoms (Osuch *et al.*, 2000) and normalizes after successful treatment via pharmacological and behavioral interventions (Mayberg *et al.*, 1999). Deep brain stimulation has been used effectively to interrupt subgenual cortex hyperactivity, resulting in amelioration of depressive symptoms (Lozano *et al.*, 2012). Subgenual cortex activity has also been shown to correlate with other autonomic markers associated with depression including vagal nerve activity and cardiac responses (Lane *et al.*, 2013), suggesting that subgenual activity is involved not only in the psychological and neurological aspects of the experience of symptoms, but also the peripheral physiological manifestations of those symptoms.

The subgenual cortex is located in close proximity to the hypothalamus in both the human and non-human primate brains. Anatomical tract tracing studies in non-human primates have demonstrated that there are direct reciprocal projections between the subgenual cortex and the hypothalamus (Ongür *et al.*, 1998a; Rempel-Clower and Barbas, 1998; Veazey *et al.*, 1982). Diffusion tensor imaging studies in humans suggest that these same pathways are conserved in humans (Johansen-Berg *et al.*, 2008). The existence of these anatomical pathways suggests direct neural communication between the subgenual cortex and the hypothalamus.

There is also evidence for indirect cortisol-mediated influence of the hypothalamus on the subgenual cortex. Corticosteroid receptors are expressed throughout the frontal cortex, including the subgenual cortex and hypothalamus (Patel *et al.*, 2000). Activity in the subgenual cortex has been shown to be modulated by exogenous cortisol (Sudheimer *et al.*, 2013). Taken together, this evidence suggests that the hypothalamus could be exerting influence over the subgenual cortex by way of direct neural connections and also indirectly via the HPA axis. As both HPA axis activity and subgenual cortex activity are closely linked with depression, we theorized that alterations in neural and/or neuroendocrine communication between the hypothalamus and the subgenual cortex could be directly driving disease-related processes in patients with non-psychotic major depression (NPMD) and depression with psychotic symptoms (PMD).

The current study was designed to determine if communication between the hypothalamus and the subgenual cortex is disturbed in patients with NPMD or PMD. To do so, a fMRI study was designed to measure resting-state functional connectivity between the hypothalamus and subgenual cortex activity in healthy participants, patients with NPMD, and patients with PMD. Resting-state func-

tional connectivity is a measure of the synchronization of discrete brain regions in low-frequency oscillations of the blood-oxygen level-dependent fMRI signal.

The main *a priori* hypothesis was that patients with major depression would have degraded hypothalamic resting-state functional connectivity with the subgenual cortex and that these disruptions would be associated with HPA axis dysregulation and greater severity of depressive symptoms. Patients with PMD were hypothesized to have the most degraded connectivity, the greatest cortisol dysregulation, and the most severe symptoms. In addition to resting-state fMRI, all participants also completed standard clinical scales to quantify depression severity and hourly overnight blood draws to characterize the circadian rhythm of cortisol secretion.

## MATERIALS AND METHODS

### Participants

A total of 113 participants (ages 18–70 years of age, Table 1) were recruited from the local community, hospitals, print advertisements, radio advertisements, and treatment clinics via referrals. All participants gave written informed consent in accordance with local Stanford University internal review board guidelines. A total of 13 of these patients were excluded from analysis due to motion artifacts, susceptibility artifacts or other artifacts that affected the quality of the neuroimaging data. The final study groups consisted of 100 participants: 39 healthy controls, 39 depressed patients without psychotic symptoms (NPMD), and 22 depressed patients with psychotic symptoms (PMD). One of the final healthy control participants was also missing overnight cortisol measures. Therefore, analyses that include cortisol samples had 38 healthy participants.

Patients were diagnosed using the Structured Clinical Interview for DSM-IV criteria. All depressed patients (NPMD and PMD) were required to have a minimum score of 18 or higher on the Hamilton Depression Ratings scale (Hamilton, 1960) and a minimum score of 6 or higher on the seven-item version of the endogenomorphic depression subscale (Thase *et al.*, 1983).

Patients with PMD were required to meet DSM-IV criteria for major depression with psychotic features at the time of the study and have active positive symptoms. These symptoms were quantified using the positive symptoms subscale of the Brief Psychiatric Ratings Scale (BPRS; Rhoades and Overall, 1988). PMD patients were all outpatients but under close supervision from their physician. All participants were excluded if they were pregnant, lactating, using illicit drugs, or abusing alcohol during the week before the study. Healthy control participants were excluded based on any history of axis I or II psychiatric disorders. Depressed patients were also excluded if they had electroconvulsive therapy within 4 months, were suicidal, or had a history of substance abuse within 6 months of the study. For ethical reasons, all patients continued any current psychiatric medication during the study but were required to maintain a stable medication regimen for at least 1 week before the study. Amongst both depressed patient groups, there were some patients with secondary (comorbid) psychiatric disorders (see Table 2).

**Table 1** Means and Standard Errors (in Parentheses) for Each Group Are Listed Along with Significant Effects Observed in Omnibus/Follow-up Tests from a Multivariate ANOVA

	Healthy (N = 39)	NPMD (N = 39)	PMD (N = 22)	Significant effects
<i>Hypothalamic connectivity with subgenual cortical regions</i>				
BA24	512.4 (116.62)	140.7 (80.22)	53.36 (173.23)	None: trend toward healthy > PMD, $p = 0.051$
BA25	739.04 (89.28)	550.26 (100.51)	545.94 (136.86)	Age: $p = 0.031$
BA32	631.04 (131.65)	144.47 (76.66)	103.71 (173.17)	Group: healthy > PMD, $p = 0.043$
<i>Cortisol</i>				
Average 1800–0100 hours, $\mu\text{g/dl}$	3.49 (0.22)	4.05 (0.3)	5.16 (0.7)	Group: healthy > PMD, $p = 0.040$
<i>Symptoms</i>				
Depressive symptoms (HAM-D)	0.49 (0.14)	23.28 (0.54)	29.86 (1.16)	Group: PMD > NPMD > healthy—PMD > NPMD, $p \leq 0.001$ , NPMD > healthy, $p \leq 0.001$
Endogenomorphic symptoms (Thase)	0.08 (0.04)	8.21 (0.25)	9.5 (0.44)	Group: PMD > NPMD > healthy—PMD > NPMD, $p = 0.017$ , NPMD > healthy, $p \leq 0.001$
Psychiatric symptoms (BPRS)	18.46 (0.17)	32.77 (0.51)	47.5 (1.57)	Group: PMD > NPMD > Healthy—PMD > NPMD, $p < 0.001$ , NPMD > Healthy, $p \leq 0.001$
Psychotic symptoms (BPRS-positive symptoms)	4.08 (0.06)	4.18 (0.08)	10.41 (0.72)	Group: PMD > (NPMD = Healthy) —PMD > NPMD, $p \leq 0.001$
Number of past episodes <sup>a</sup>	0 (0)	6.58 (1.09)	7.16 (1.43)	Group: (PMD = NPMD) > healthy, NPMD > healthy $p < 0.004$ , PMD > healthy $p < 0.001$
Duration of current episode (weeks) <sup>b</sup>	0 (0)	121.83 (33.36)	170.94 (0.97)	Group: (PMD = NPMD) > healthy —NPMD > healthy $p < 0.02$ , PMD > healthy $p < 0.01$ ,
<i>Demographics</i>				
Age	34.13 (2.24)	42.23 (2.21)	39.73 (2.86)	Group: NPMD > (healthy = NPMD)—NPMD > healthy $p = 0.01$
Education	15.88 (0.32)	15.08 (0.3)	15.55 (0.65)	None
Sex (% male/female)	38.5/61.5%	28.2/71.8%	40.0/59.1%	None

Abbreviations: NPMD, non-psychotic major depression; PMD, psychotic major depression.

<sup>a</sup>NPMD (N = 23), PMD (N = 19).

<sup>b</sup>NPMD (N = 29), PMD (N = 18).

Follow up tests were *t*-tests or  $\chi^2$  (for gender).

## Protocol

A between-groups design was used. Each participant in the three groups completed a comprehensive psychiatric assessment, hourly overnight blood draws from 1800 to 0900 hours to measure circadian cortisol secretion, and a fMRI scanning session.

fMRI volumes were collected over a 5-min scan while participants rested with their eyes closed inside a 3T GE Signa scanner (General Electric Corporation) equipped with a radio frequency head coil. These volumes were echo planar images with a 2-mm isotropic voxel dimensions. Functional images were slice-time corrected, realigned (motion corrected), co-registered, normalized to a standard template, and smoothed with a 8-mm Gaussian kernel using SPM8. Smoothing was designed to be sensitive subgenual cortex target clusters. The functional time series was also detrended using a voxel-wise global signal correction (Macey *et al*, 2004), and band-pass filtered to isolate the typical functional connectivity frequency range (0.008–0.1 Hz) from high and low-frequency noise.

Functional connectivity was assessed with a region of interest (ROI), seed-based approach using the statistical parametric mapping (SPM8) platform and Matlab (Mathworks, Natick, Massachusetts). A preprocessed time series was extracted from the hypothalamus, as defined in the Anatomical Automatic Labeling atlas (Tzourio-Mazoyer *et al*, 2002), and verified using other available online (Sudheimer *et al*, n.d.) and print atlases (Damasio, 1995; Mai *et al*, 1997). This subject-specific hypothalamus time series was then used to test for correlated time series across the whole brain using a simple regression first-level model. For each subject, a beta and a contrast image was created based on this regression. The voxel values in these contrast images indicate the strength of connectivity between the hypothalamus and each voxel in the entire brain. These first-level contrast images were then used as input into a second-level (random effects) model that tested for between-group differences in the strength of hypothalamic functional connectivity.

In addition to testing for group differences in hypothalamus connectivity across the whole brain, we had an *a priori*

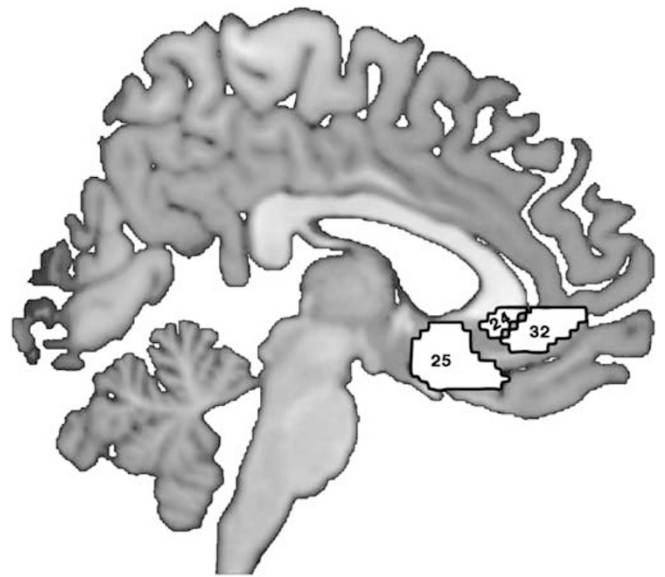
**Table 2** The Number of Participants Who Were Taking Medications Are Listed by Type and Combination

	Group		
	Healthy (N = 39)	NPMD (N = 39)	PMD (N = 22)
<i>Medication classes and combinations</i>			
No medications	39	16	3
Antidepressant only	0	19	3
Antipsychotic only	0	0	0
Axiolytic only	0	0	0
Antidepressant + antipsychotic	0	0	7
Antidepressant + axiolytic	0	4	1
Antipsychotic + axiolytic	0	0	1
Antidepressant + antipsychotic + axiolytic	0	0	7
<i>Psychiatric diagnoses and co-morbidities</i>			
Bipolar	0	1	2
Major depression	0	39	22
Psychotic major depression	0	0	22
Schizophrenia	0	0	0
Schizoaffective	0	0	0
Panic disorder	0	1	3
Agoraphobia	0	2	2
Social phobia	0	3	5
Specific phobia	0	5	2
Obsessive compulsive	0	0	4
Post-traumatic stress	0	8	4
Generalized anxiety disorder	0	3	3

Abbreviations: NPMD, non-psychotic major depression; PMD, psychotic major depression.

Some patients present with multiple diagnoses.

hypothesis of disrupted connectivity between the hypothalamus and the subgenual cortex. However, the exact regions of the subgenual cortex that have been implicated in depression are somewhat variable. Across a number of studies the regions implicated generally conform to parts of (but not the entire extent of) Brodmann areas (BA) 25, 24, 32 and the subcallosal gyrus (Hamani *et al*, 2011). Therefore, smoothed binary masks were created from these regions using the spatial definitions provided by the Automatic Anatomical Labeling atlas (Tzourio-Mazoyer *et al*, 2002). However, as BA24 and BA32 extend dorsally beyond the subgenual regions that are generally implicated in major depression, the masks were restricted to midline frontal regions that are ventral to the genu of the corpus callosum ( $x = -20$  to  $+20$ ,  $y = 0$  to  $+50$ ,  $z < 0$ ) (Figure 1). These ROIs were used in small volume corrections (SVC), as a compliment to the whole-brain hypothalamus connectivity analysis. In order for a SVC, a cluster of voxels was considered to be significant if it met an initial uncorrected threshold of  $p < 0.001$  and a cluster threshold of 10 voxels, and then also met a family-wise corrected threshold of  $p < 0.05$  within any of the small volumes. SVC FWE corrections were made within each subregion.



**Figure 1** Anatomically defined regions of interest (ROI) were used for Brodmann areas (BA) 24, BA25, and BA32. The ROIs were smoothed to match to spatial resolution of the functional connectivity data and then converted to binary mask images. The spatial extent was also limited to reflect the typical boundaries of subgenual cortex (ie, limited to cortex inferior to the genu of the corpus callosum).

Measures of the circadian rhythm of cortisol secretions were collected overnight at hourly intervals from 1800 to 0800 hours, for a total of 15 blood samples. Some of these data were reported in previous publications. Blood (8 ml/sample) was drawn from an indwelling venous catheter. Plasma was immediately separated from whole blood by centrifugation and then stored at  $-70^{\circ}\text{C}$  until assays were performed. Plasma cortisol concentrations were measured using the Access Immunoassay System (Beckman Coulter, Chaska, MN). The sensitivity of this assay is 0.4 mcg/dl (11 nmol/l) and the precision within assays is 6.4–7.9%. Cortisol was also measured in the saliva of a subsample of 30 healthy, 36 NPMD, and 16 PMD patients using the Salivette cotton swab system (Sarstedt, Nümbrecht, Germany) and was analyzed by Salimetrics (State College, PA) using an enzyme immunoassay with a lower limit sensitivity of 0.003  $\mu\text{g/dl}$  and average intra-assay and inter-assay coefficients of variation of 3.5 and 5.1%. Two samples were collected from each participant, one before, and one after the fMRI scan. The mean of these two measures was used to estimate salivary cortisol during scanning.

### Data Analysis

**Cortisol.** To determine group differences in plasma cortisol, a repeated-measures ANOVA with linearly interpolated missing values was conducted. Since there was also an *a priori* hypotheses regarding elevated cortisol during the circadian nadir, these values were also averaged across the 1800 to 0100 hours time window, which constitutes the typical circadian nadir. These averages were compared across groups using a standard ANOVA. Salivary cortisol measures collected on a subset of participants were averaged across



the samples collected immediately before and immediately after fMRI scanning these values were also analyzed using a standard ANOVA model.

**Functional connectivity.** In addition to whole-brain and SVC analyses using SPM, we also conducted a multivariate ANOVA (MANOVA) using extracted average functional connectivity from our subgenual ROIs, plasma cortisol measures, and measures of symptom severity. This MANOVA controlled for both age and gender as factors while testing for group differences in functional connectivity, cortisol secretion, and symptom severity.

**Relationships between hypothalamic functional connectivity, cortisol, and symptoms.** Backward-stepwise multivariate linear regressions were conducted using SPSS to test for the relative contributions of age, gender, cortisol secretion, and functional connectivity measure to depressive symptoms. As variance across all groups and variance within the depressed group are both potentially informative these regressions were conducted across the whole sample, and a separate regression was also conducted just using all depressed patients (NPMD and PMD). Similar regression analyses were conducted to test the relative contributions of age, gender, cortisol secretion, and symptoms to functional connectivity disturbances.

## RESULTS

All three participant groups demonstrated positive connectivity between the hypothalamus seed region and voxels located within or adjacent to the hypothalamus. Healthy participants exhibited strong hypothalamic connectivity to the subgenual cortex, insula, hippocampus, and superior/middle temporal gyri (Table 3, Figure 2). This pattern of strong hypothalamic connectivity was reduced in the NPMD and PMD patients (Figure 2). Formal statistical group comparisons indicated clusters of voxels in which the strength of the hypothalamic connectivity to the subgenual cortex was significantly lower in both NPMD and PMD patients compared with the healthy control group (Figure 3). These reductions in connectivity in patients were significant at a FWE threshold of  $p < 0.05$  after SVC when comparing either group of patients to healthy participants (Table 3). No significant differences in hypothalamic connectivity were seen between the NPMD and PMD groups.

In addition to the whole-brain analysis, the strength of the hypothalamic functional connectivity was also averaged across each of the three ROI. These ROI-averaged values were corrected for age and gender inside a MANOVA model. Across all three ROIs hypothalamic connectivity to subgenual cortex showed a stepwise reduction when comparing across groups with healthy participants having the highest connectivity and PMD patients having the lowest (Figure 4). However, there was only a significant omnibus effect of group for the subgenual BA32 region. This effect was driven by lower connectivity in the PMD group vs the healthy group (Table 2). In BA24, the omnibus effect of group on hypothalamic connectivity was just sub-threshold for significance at  $p = 0.051$ , which was driven by

a trend toward reduced connectivity in the PMD patients relative to the healthy participants.

A significant effect of age on hypothalamic connectivity to BA25 was also observed. This effect was driven by reduced connectivity in older participants relative to younger participants (Table 2).

Cortisol secretions during the circadian nadir hours of 1800 to 0100 hours were significantly elevated in PMD patients. However, these differences did not persist throughout the whole circadian curve or into the early morning rising phase (Table 1, Figure 5). There were also no group differences observed in the sample of participants that had salivary cortisol collected at the time of scanning.

Across all participants, more severe symptoms as measured by the HAM-D total score, the endogenomorphic subscale, or the BPRS, were associated with both decreased hypothalamic connectivity to the subgenual BA32 and increased cortisol secretion during the circadian nadir. When these same analyses were conducted across the subset of only depressed patients (NPMD and PMD), only cortisol secretion was associated with increased symptoms on the HAM-D and endogenomorphic subscale (Table 4).

Across all participants, more severe disruptions in hypothalamic connectivity were negatively correlated with cortisol secretion at a trend-level significance (BA24  $r = -0.170$ ,  $p = 0.092$ ; BA25  $r = -0.179$ ,  $p = 0.077$ ; BA32  $r = -0.196$ ,  $p = 0.052$ ). However, these effects were not significant after correcting for age, gender, or symptoms in a regression analysis. Similar non-significant negative correlations were also seen when the analysis was conducted across the subset of only depressed patients.

## DISCUSSION

This study is the first whole-brain characterization of resting-state functional connectivity patterns of the hypothalamus. The findings suggest that in healthy participants there is strong hypothalamic connectivity with the subgenual cortex and other regions including the insula, superior, and middle temporal gyri. The existence of a neural circuit that could be driving these functional connectivity relationships is strongly supported by tract tracing studies in non-human primates (Johansen-Berg et al, 2008; Ongür et al, 1998a) and diffusion tensor imaging studies in humans (Johansen-Berg et al, 2008; Ongür et al, 1998b).

These data also provide the first evidence that hypothalamic connectivity with the subgenual cortex is disrupted in patients with PMD, suggesting that aspects of the pathophysiology of depression could be associated with these changes. Although only the PMD patients presented with statistically significant reduced functional connectivity, it is noteworthy that across each of the subgenual ROIs, a stepwise reduction in hypothalamic connectivity was observed with healthy controls having the strongest connectivity, followed by the NPMD patients, and PMD patients having the weakest connectivity (Figure 4).

Multiple regression models (Table 4) suggest that the large symptom differences that occur between healthy individuals and depressed individuals could be related to both excessive cortisol secretion during the circadian nadir and degraded functional connectivity between the

**Table 3** Whole-Brain and Small Volume Corrected Neuroimaging Results Show Robust Hypothalamic Connectivity with the Subgenual Cortex, the Insula, Temporal Lobe, and Several Other Subcortical Regions in Healthy Participants

Group hypothalamus functional connectivity							
Group	Region/s	Peak voxel (x, y, z) <sup>a</sup>	Cluster <sup>b</sup>		P-value (unc.) <sup>c</sup>	P-value (FWE-SVC) <sup>d</sup>	P-value (FWE-Whole Brain) <sup>e</sup>
<i>Healthy &gt; 0</i>							
	Anterior cingulate, subgenual cortex, subcallosal gyrus, rectal gyrus, parahippocampal gyrus, lingual gyrus, orbital gyrus, inferior frontal gyrus, middle frontal gyrus, superior frontal gyrus, medial frontal gyrus, culmen, lentiform nucleus, thalamus, caudate, hippocampus, amygdala, BA10/11/13/19/24/25/27/28/30/32/34/35/36/37/38/47, mammillary body, globus pallidus, putamen, hypothalamus	2, 0, -10	9903	Inf	<0.000001	<0.001	<0.001
	Insula, middle temporal gyrus, superior temporal gyrus, BA13/21/22/38	48, -6, -14	202	5.01	<0.000001	—	0.013
	Cerebellum: declive of vermis, nodule, pyramis, culmen	-2, -62, -20	117	4.15	0.000017	—	—
	Cingulate gyrus, precuneus, BA31	20, -54, 26	130	3.80	0.000073	—	—
	Insula, superior temporal gyrus, BA13/22	-42, 0, -2	36	3.57	0.000181	—	—
	Insula, precentral gyrus	46, 0, 16	15	3.50	0.000235	—	—
	Frontal lobe white matter	26, 18, 36	21	3.39	0.000352	—	—
	Superior temporal gyrus	42, -52, 20	17	3.37	0.000370	—	—
	Temporal lobe white matter	-26, -60, 22	11	3.22	0.000643	—	—
<i>NPMD &gt; 0</i>							
	Anterior cingulate, subgenual cortex, subcallosal gyrus, rectal gyrus, parahippocampal gyrus, orbital gyrus, superior temporal gyrus, inferior frontal gyrus, middle frontal gyrus, superior frontal gyrus, medial frontal gyrus, lentiform nucleus, caudate, amygdala, ba10/11/13/24/25/28/32/34/38/47, globus pallidus, putamen, hypothalamus	2, 0, -12	4852	7.67	<0.000001	<0.001	<0.001
	Middle occipital gyrus	-34, -72, 0	108	3.99	0.000034	—	—
	Middle temporal gyrus, superior temporal gyrus, BA39	-30, -68, 20	115	3.93	0.000042	—	—
	Superior temporal gyrus, BA20/36/38	30, 10, -50	81	3.81	0.000069	—	—
	Fusiform gyrus, parahippocampal gyrus, culmen, BA20/36	-30, -34, -24	27	3.60	0.000161	—	—
	Middle temporal gyrus	-66, -70, 2	29	3.59	0.000165	—	—
	Parahippocampal gyrus, BA19	-22, -44, -4	39	3.40	0.000332	—	—
	Thalamus, pulvinar	-16, -36, 8	14	3.31	0.000470	—	—
<i>PMD &gt; 0</i>							
	Midbrain, anterior cingulate, subcallosal gyrus, uncus, rectal gyrus, parahippocampal gyrus, inferior frontal gyrus, medial frontal gyrus, lentiform nucleus, thalamus, ba11/25/34/47, medial dorsal nucleus, ventral lateral nucleus, anterior nucleus, mammillary body, medial globus pallidus, putamen, hypothalamus	0, 0, -12	1773	Inf	<0.000001	0.008	<0.001
	Cerebellar tonsil, pons	18, -42, -44	11	3.35	0.000400597	—	—
	Lentiform nucleus, thalamus, putamen	26, -10, 8	13	3.18	0.000724495	—	—

**Table 3 (Continued)**

Group hypothalamus functional connectivity							
Group	Region/s	Peak voxel (x, y, z) <sup>a</sup>	Cluster <sup>b</sup>	P-value (unc.) <sup>c</sup>	P-value (FWE-SVC) <sup>d</sup>	P-value (FWE-Whole Brain) <sup>e</sup>	
Group differences in hypothalamus functional connectivity							
Group comparison							
<i>Healthy &gt; PMD</i>							
	Precuneus, BA7	16, -64, 42	34	3.72	0.000098	—	
	Precuneus	-26, -60, 22	50	3.65	0.000131	—	
	Superior frontal gyrus, medial frontal gyrus, BA10	10, 62, -6	33	3.50	0.000236	—	
	Parahippocampal gyrus, BA19	-36, -52, -4	42	3.46	0.000274	—	
	Medial frontal gyrus	36, 46, -2	18	3.45	0.000285	—	
	Subgenual cortex, anterior cingulate, medial frontal gyrus, BA10/11/32	2, 44, -12	172	3.42	0.000314	0.029	
	Precuneus, BA7	-10, -58, 56	14	3.31	0.000461	—	
	Medial frontal gyrus	-14, 40, -8	14	3.28	0.000520	—	
	Cingulate gyrus, BA31	18, -40, 30	12	3.24	0.000592	—	
<i>PMD &gt; healthy</i>							
	Cuneus, BA18	0, -102, 10	80	3.79	0.000075	—	
	Lingual gyrus, cuneus, BA17/18	14, -104, -10	189	3.71	0.000104	—	
	Thalamus, anterior nucleus, medial dorsal nucleus	-2, -6, 12	93	3.58	0.000174	—	
	Middle frontal gyrus, inferior frontal gyrus, BA9/46	-44, 16, 28	63	3.57	0.000178	—	
	Lingual gyrus, BA17/18	-6, -104, -10	34	3.31	0.000462	—	
<i>Healthy &gt; NPMD</i>							
	Superior temporal gyrus, middle temporal gyrus, BA13/21/22/38	48, -6, -14	130	4.54	0.000003	—	
	Insula, inferior frontal gyrus, BA13/44/45	42, 18, 12	98	3.91	0.000047	—	
	Medial frontal gyrus, BA6	-12, -6, 56	24	3.72	0.000099	—	
	Middle frontal gyrus, superior frontal gyrus, BA6	16, -6, 66	38	3.72	0.000101	—	
	Superior temporal gyrus, BA6/13/22	-44, 0, -2	112	3.70	0.000106	—	
	Subgenual cortex, anterior cingulate, medial frontal gyrus, BA10/11/24/32	4, 46, -12	99	3.58	0.000174	0.018	
	Superior temporal gyrus, precentral gyrus, BA6/13/22/44	46, -2, 6	62	3.51	0.000222	—	
	Cingulate gyrus, superior frontal gyrus, medial frontal gyrus, BA6/32	20, 6, 52	20	3.46	0.000269	—	
	Inferior frontal gyrus	-34, 36, 4	10	3.44	0.000295	—	
	Midbrain, thalamus, hypothalamus	0, -12, -4	17	3.38	0.000368	—	
	Insula, claustrum	34, -4, -2	22	3.36	0.000385	—	
	Superior frontal gyrus	-8, 14, 72	17	3.35	0.000404	—	

**Table 3 (Continued)**

Group hypothalamus functional connectivity							
Group	Region/s	Peak voxel (x, y, z) <sup>a</sup>	Cluster <sup>b</sup>		P-value (unc.) <sup>c</sup>	P-value (FWE-SVC) <sup>d</sup>	P-value (FWE-Whole Brain) <sup>e</sup>
	Cingulate gyrus	− 8, − 8, 32	11	3.32	0.000443	—	—
	Insula, inferior frontal gyrus, BA13/47	− 36, 14, − 8	14	3.30	0.000489	—	—
<i>NPMD &gt; healthy</i>							
	Cuneus, precuneus, BA7/19/31	− 28, − 74, 24	58	3.60	0.000162	—	—
	Middle occipital gyrus, BA19	44, − 74, − 14	12	3.28	0.000523	—	—
<i>NPMD &gt; PMD</i>							
	Parahippocampal gyrus, BA19/37	− 34, − 50, − 8	88	3.71	0.000104	—	—
	Parahippocampal gyrus, BA36	− 32, − 30, − 24	18	3.62	0.000146	—	—
	Temporal/parietal/occipital junction	− 28, − 64, 24	23	3.28	0.000520	—	—
<i>PMD &gt; NPMD</i>							
	Lentiform nucleus, lateral globus pallidus, putamen	− 22, − 8, 4	40	3.50	0.000228	—	—
	Posterior cingulate, BA29	2, − 48, 8	48	3.45	0.000280	—	—
	Superior frontal gyrus, BA10	− 30, 70, 4	24	3.45	0.000283	—	—
	Thalamus	− 4, − 6, 8	21	3.44	0.000295	—	—
	Cuneus	4, − 102, 12	15	3.34	0.000413	—	—
	Inferior frontal gyrus, BA45/46	− 48, 26, 20	15	3.34	0.000415	—	—
	Inferior frontal gyrus, BA47	− 48, 34, − 12	15	3.24	0.000608	—	—

Abbreviations: FWE, family-wise error; NPMD, non-psychotic major depression; PMD, psychotic major depression; SVC, small volume correction.

<sup>a</sup>Peak voxel coordinate in standard MNI space. Clusters of larger sizes can cover regions outside the region that houses the peak voxel value. Regions containing 10 or more significant voxels are listed in the Region/s column.

<sup>b</sup>Cluster size threshold was set to 10 voxels.

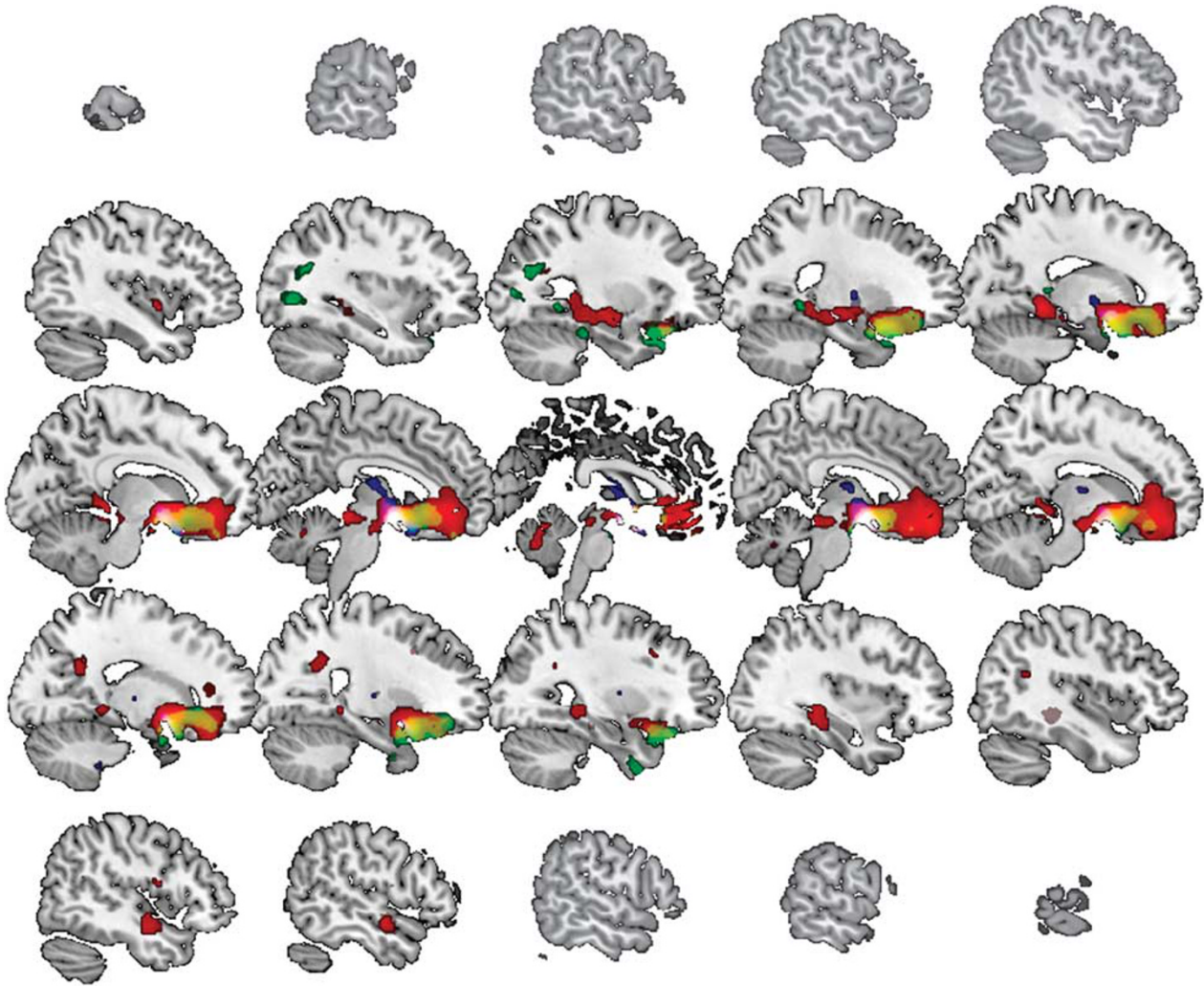
<sup>c</sup>*p*-values uncorrected for multiple comparisons.

<sup>d</sup>Family-wise error correction was applied within the *a priori* regions of interest as a small volume correction (SVC).

<sup>e</sup>Family-wise error correction was applied.

Voxel-wise group comparisons indicate lower hypothalamic connectivity to the subgenual cortex in the depressed patients.





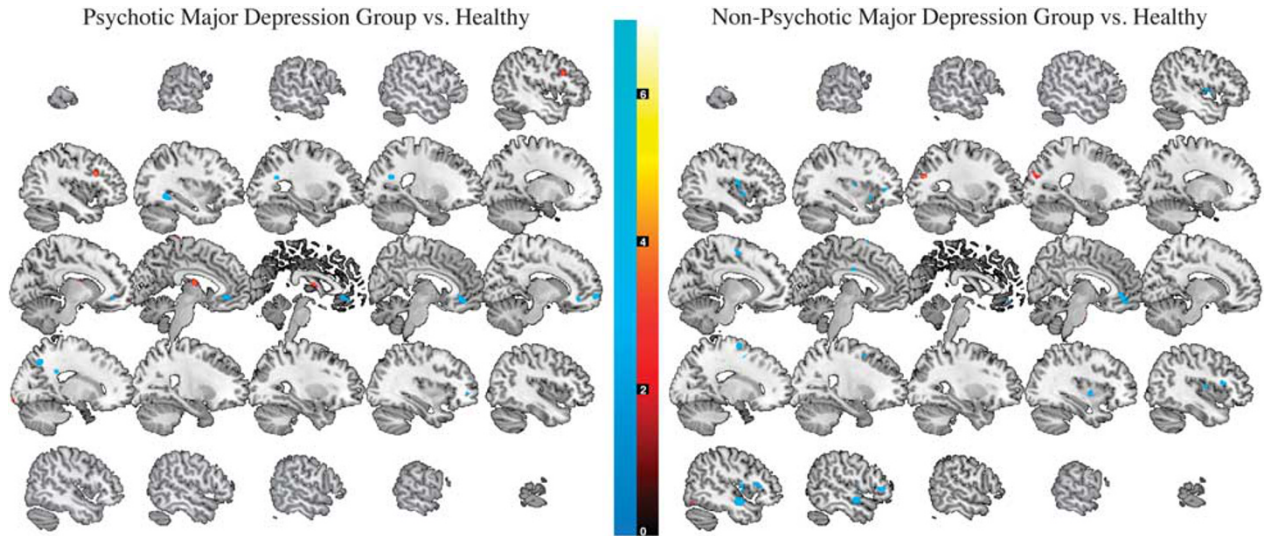
**Figure 2** Healthy group, red; the non-psychotic major depression group (NPMD; green); psychotic major depression group, blue; healthy and NPMD overlap, yellow; healthy and psychotic major depression overlap, violet; NPMD and psychotic major depression overlap, cyan; and all three groups overlap, white. Conjunction maps demonstrate shared and differential spatial patterns of hypothalamic connectivity. The healthy group demonstrated significant hypothalamic connectivity to the subgenual cortex, insula, middle, and superior temporal gyri. The healthy group and the NPMD group showed some common connectivity patterns but a reduced spatial extent of hypothalamic connectivity with the subgenual cortex. The spatial extent of this connectivity is further reduced in the psychotic major depression group. All functional maps are subject to a  $p < 0.001$  (unc.) voxel threshold and a cluster threshold of  $k = 10$  for display purposes (see Table 2 for SVC, FWE-corrected statistics).

hypothalamus and BA32. However, these models also suggest that degradations in the hypothalamic functional connectivity patterns do not explain why some NPMD or PMD patients experience more severe symptoms than others. Variance between depressed individuals on cortisol secretion was more associated with these smaller changes in symptomatology.

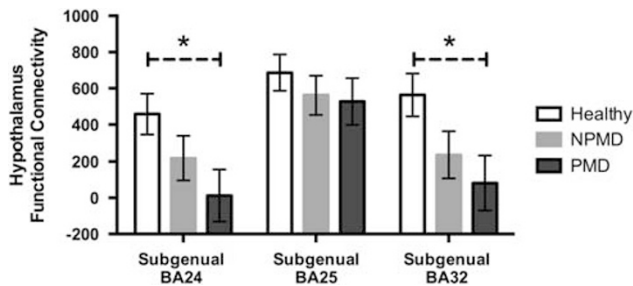
Lower hypothalamic connectivity with increased age is particularly notable (BA25, Table 2) as older adults generally have worse regulation of the HPA axis, and higher rates of cortisol secretion across the circadian rhythm. In our sample, cortisol secretion and age were fairly co-linear, which reduced our ability to discern the effects of age from the effects of cortisol secretion on hypothalamic connectivity to the subgenual cortex. When age is not included as a covariate in the multivariate models, significantly lower hypothalamic connectivity to BA25 was seen in the patient

groups. However, the data that we present cannot resolve if age, cortisol, or both could be the main contributing factor to lower hypothalamic connectivity with BA25. Future studies with a narrower range of ages or 1:1 subject matching could resolve this ambiguity.

Previous studies have closely linked hyperactivation of the subgenual cortex with depression (Mayberg *et al*, 1999) and have shown that this hyperactivation normalizes with successful treatment (Mayberg *et al*, 1997). Prior work has also reported increased subgenual cortex connectivity with the default mode network (Greicius *et al*, 2007), suggesting that ramped-up subgenual activity could be a driving force behind the disruptions in hypothalamic connectivity that we observe. Glucocorticoid receptors are expressed throughout the frontal cortex in humans (Watzka *et al*, 2000), and cortisol can affect the emotion-related activation patterns of the subgenual cortex (Sudheimer *et al*, 2013).



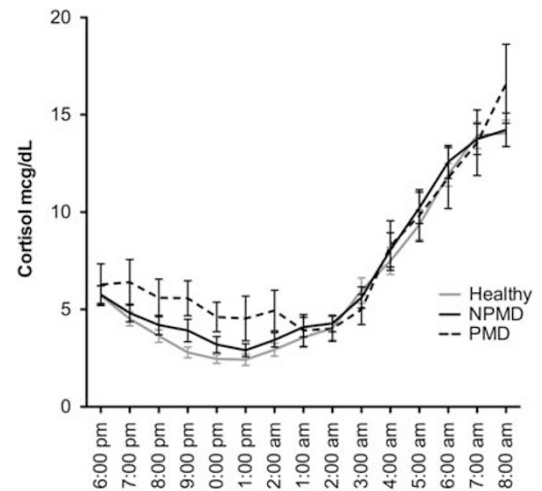
**Figure 3** Whole-brain group comparison maps indicate that hypothalamic functional connectivity to the subgenual cortex is decreased in both the psychotic major depression and non-psychotic major depression groups (3rd row, 4th column in both maps). Functional maps are subject to a  $p < 0.001$  (unc.) voxel threshold and a cluster threshold of  $k = 10$  for display purposes (see Table 2 for SVC, FWE-corrected statistics, and comparisons between psychotic and non-psychotic depression groups).



**Figure 4** Age/gender corrected region of interest extractions represent connectivity between the hypothalamus seed and each of the subgenual regions averaged across all voxels. Hypothalamus connectivity is decreased in both subgenual regions BA24 and BA32, but not BA25.

These findings when taken together suggest a model of how subgenual cortex, the hypothalamus, and cortisol signaling could be interacting and affecting each other's functioning. The hypothalamus can exert control over the subgenual cortex via direct neural projections as well as indirectly through HPA-axis activation and subsequent cortisol modulation of the subgenual cortex. The subgenual cortex could also exert influence on hypothalamic and HPA axis activity via projections to the paraventricular nucleus of the hypothalamus, which is responsible for initiating corticotropin-releasing-hormone release (Rempel-Clower and Barbas, 1998). Finally, cortisol via signaling at its receptors is able to modulate both hypothalamic and subgenual cortex activity through receptors expressed in those regions.

Thus, there are many potential routes to disrupted functional connectivity between the hypothalamus and the subgenual cortex. Cortisol is capable of inhibiting both hypothalamic output and subgenual cingulate functions, but could be doing so with differential efficacy based on



**Figure 5** Cortisol secretion is elevated in the psychotic major depression group during the 1800–0100-hour window, which typically represents the circadian nadir.

regional rates of receptor downregulation in depression, leading to desynchronization of the regions.

A disruption in the communication or regulatory balance between the hypothalamus, subgenual cortex, and HPA axis activity could be resulting in downstream effects on symptoms. However, the experience of symptoms could also be driving connectivity and HPA axis changes. The data presented here suggest that disruptions in hypothalamic connectivity to the subgenual cortex and cortisol hypersecretion are both associated with overall increases in depressive symptoms but that cortisol may be more closely linked with symptom severity once the disease state is

**Table 4** All Backwards Stepwise Multivariate Regression Models Initially Contain Age, Gender, Cortisol, and Hypothalamic Connectivity with BA24, BA25, and BA32

Dependant variable	Groups in model	Variable	Standardized beta	T-value	Significant effects
HAM-D 21	All participants	Age	0.187	1.935	0.056
		Cortisol	0.213	2.227	0.028
		BA32	-0.223	-2.311	0.023
	All depressed	Gender	-0.242	-1.993	0.051
		Cortisol	0.295	2.43	0.018
HAM-D (Endog. Subscale)	All participants	Age	0.209	2.206	0.03
		Cortisol	0.238	2.543	0.013
		BA32	-0.228	-2.404	0.018
	All depressed	Gender	-0.288	-2.546	0.014
		Cortisol	0.41	3.616	0.001
BPRS	All participants	Cortisol	0.207	2.147	0.034
		BA32	-0.277	-2.868	0.005
	All depressed	Gender	-0.215	-1.691	0.096

Abbreviations: BPRS, Brief Psychiatric Ratings Scale; HAM-D, Hamilton Depression Ratings Scale.

Variance in symptoms across all participants was explained by higher cortisol secretion and lower hypothalamic connectivity to BA32. Variance in symptoms across the depressed groups, all of which had high levels of symptoms (see also Table 1) was explained by higher cortisol secretion and gender.

established. Follow-up studies are needed to determine the causal relationships between hypothalamic connectivity, cortisol secretion/regulation, and symptom severity. Additional depression subtypes with HPA-axis dysregulation such as the melancholic subtype, may also have similar or even greater functional connectivity disruptions between the hypothalamus and the subgenual cortex.

This study had several limitations. First, it is difficult to recruit patients with PMD as it is a relatively rare depression subtype representing 18.5% of patients who meet the criteria major depression and 0.4% of the general population (Ohayon, 2002). Adding to this challenge is that psychotic symptoms tend to resolve quickly after treatment with antipsychotic medication. Therefore, it was not feasible to recruit PMD patients with active psychotic symptoms who were either drug free or even patients who were on standardized medication regimes. However, to limit confounding influences of switching/reducing psychiatric medication regimes and potential ethic concerns of having seriously ill medication-free patients, we required all participants to have stable medications for at least 1 week before participation. These medications could be impacting functional connectivity in unexpected ways; however, additional studies will be needed to amass a sample size of PMD patients that will provide the statistical power necessary to delineate the effects of specific medication on PMD functional connectivity patterns. Another limitation is that the PMD group had higher total symptoms scores on the HAM-D and the endogenomorphic subscale as compared with depressed patients without psychotic symptoms (Table 1). These differences arise from several items on the HAM-D that exploit core symptoms of psychosis, such as 'unusual thought content'. No significant connectivity or cortisol secretion differences were observed between NPMD and PMD regardless of if these total symptoms scores were included as a covariate or not.

## CONCLUSIONS

This study provides the first descriptions of functional connectivity between the hypothalamus and subgenual cortex and also the first evidence that this relationship is perturbed in patients with PMD. The main *a priori* hypotheses in this study were only partially confirmed. Only some patients (PMD subtype) can be confirmed as having degraded hypothalamic resting-state functional connectivity with the subgenual cortex. PMD patients did present with the most degraded connectivity, greatest cortisol dysregulation, and the most severe symptoms. Both degraded connectivity and cortisol dysregulation were also associated with greater symptoms although cortisol dysregulation may be more associated with differences symptom differences amongst depressed patients, whereas degraded connectivity may be more associated with the symptom differences between healthy patients and depressed patients.

Longitudinal studies are required to address if these connectivity disruptions resolve alongside clinical improvements or predict relapse rates. Additional follow-up studies are needed to determine the hypothalamus-subgenual cortex-HPA axis circuit's potential contribution to the symptoms of depression.

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Dr Reiss has received Consulting fee from Novartis Pharmaceuticals. Dr Schatzberg has served as a consultant to Bay City Capital, BrainCells, CeNeRx, Cervel, Depomed, Eli Lilly, EnVivo (Forum), Genentech, Gilead, Jazz, Lundbeck/Takeda, McKinsey, Merck\*, MSI, Naurex, Neuronetics, Novadel, Pfizer\*, PharmaNeuroBoost\*, Sunovion, Synosia, and Xhale. Dr Schatzberg has had equity in Amnestix, BrainCells, CeNeRx, Cervel, Corcept\* (co-founder), Delpor, Forest Labs, Merck\*, Neurocrine, Novadel, Pfizer\*,



PharmaNeuroBoost, Seattle Genetics\*, Somaxon, Synosia, Titan, and Xhale\*. He is a named inventor on pharmacogenetic use patents on glucocorticoid antagonists and on prediction of antidepressant response. Dr Schatzberg has also received speaking fees from Merck. \*Greater than \$10,000/year in income or current equity value. The remaining authors declare no conflict of interest.

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