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REGULAR RESEARCH ARTICLE

Cortisol Stress Response and in Vivo PET Imaging of Human Brain Serotonin 1A Receptor Binding

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Abstract

Background: Abnormalities in the hypothalamic-pituitary-adrenal axis, serotonergic system, and stress response have been linked to the pathogenesis of major depressive disorder. State-dependent hyper-reactivity of the hypothalamic-pituitary-adrenal axis is seen in major depressive disorder, and higher binding to the serotonin 1A receptor is observed as a trait in both currently depressed and remitted untreated major depressive disorder. Here, we sought to examine whether a relationship exists between cortisol secretion in response to a stressor and serotonin 1A receptor binding throughout the brain, both in healthy controls and participants with major depressive disorder.

Methods: Research participants included 42 medication-free, depressed subjects and 31 healthy volunteers. Participants were exposed to either an acute, physical stressor (radial artery catheter insertion) or a psychological stressor (Trier Social Stress Test). Levels of serotonin 1A receptor binding on positron emission tomography with [^{11}C]WAY-100635 were also obtained from all participants. The relationship between [^{11}C]WAY-100635 binding and cortisol was examined using mixed linear effects models with group (major depressive disorder vs control), cortisol, brain region, and their interactions as fixed effects and subject as a random effect.

Results: We found a positive correlation between post-stress cortisol measures and serotonin 1A receptor ligand binding levels across multiple cortical and subcortical regions, independent of diagnosis and with both types of stress. The relationship between [^{11}C]WAY-100635 binding and cortisol was homogenous across all a priori brain regions. In contrast, resting cortisol levels were negatively correlated with serotonin 1A receptor ligand binding levels independently of diagnosis, except in the RN. There was no significant difference in cortisol between major depressive disorder participants and healthy volunteers with either stressor. Similarly, there was no correlation between cortisol and depression severity in either stressor group.

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Significance Statement

The relationship between the body's response to stress and the function of serotonin in the brain has been a subject of interest in depression research. Several studies have examined this by manipulating the body's stress response system externally—either by administering medications, such as steroids, or by removing adrenal glands surgically. The data resulting from these studies has been mixed and inconclusive. Here, we show human data from healthy volunteers and participants with major depression. We studied the body's self-generated stress response, cortisol levels, to 2 different types of stressors: a physical and a psychological stressor. We found that with both stressors, greater cortisol levels are associated with higher levels of serotonin 1A receptor binding throughout the brain. There is also an inverse association between resting cortisol levels and receptor binding. Both findings were independent of diagnosis. This suggests that healthy volunteers and depressed subjects are biologically on a continuum, and that individuals with a more pronounced stress response tend to have higher levels of serotonin 1A receptor throughout the brain. Our data add to the literature by demonstrating a link between acute stress responses and serotonin 1A receptor availability in humans.

Conclusions: This study suggests that there may be a common underlying mechanism that links abnormalities in the serotonin system and hypothalamic-pituitary-adrenal axis hyper-reactivity to stress. Future studies need to determine how hypothalamic-pituitary-adrenal axis dysfunction affects mood to increase the risk of suicide in major depression.

Keywords: cortisol, PET imaging, serotonin, stress

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis and the serotonin system have a complex bidirectional relationship (Meijer and de Kloet, 1994; Porter et al., 2004). This relationship is of potential importance in the pathogenesis of mood disorders, because major depressive episodes are associated with trait abnormalities in the serotonin system, including 5-HT_{1A} receptor upregulation (Parsey et al., 2006a), and with hyperactive HPA axis responses to stress, which are state dependent (Cowen, 2010; Stetler and Miller, 2011). More severe depressive episodes, such as those characterized by psychomotor agitation or psychotic features, have more severely dysregulated HPA axis function as indicated by heavier adrenal glands, higher levels of corticotropin releasing factor (CRF) in brain tissue and cerebrospinal fluid, lower CRF receptor binding in prefrontal cortex postmortem, a blunted cortisol suppression response to dexamethasone, and greater cortisol release both at baseline and in response to social stressors (Lindy et al., 1985; Brown et al., 1986; Nemeroff et al., 1988; Arató et al., 1989; Pfennig et al., 2005; Mann et al., 2006; Melhem et al., 2016). Genetic and epigenetic associations with enhanced HPA axis stress responses have also been observed in major depressive disorder (MDD) and in those reporting early-life stress, which is a risk factor for MDD (McGowan et al., 2009; Coplan et al., 2011; Yin et al., 2016).

The relationship between markers of serotonergic tone and HPA axis function has been extensively studied. Cortisol induces tryptophan 2,3-dioxygenase, which metabolizes L-tryptophan, thereby decreasing L-tryptophan availability for serotonin synthesis (Badawy et al., 1995). Animal work has demonstrated that adrenalectomized rats show increased post-synaptic serotonin-1A (5-HT_{1A}) receptor binding in the hippocampus, whereas chronic treatment with corticosterone reduces expression, binding, and function of 5-HT_{1A} receptors in hippocampal fields (Martire et al., 1989; Mendelson and McEwen, 1992; Chalmers et al., 1993; Kuroda et al., 1994; Meijer and de Kloet, 1994; Laaris et al., 1995; Zhong and Ciaranello, 1995; Le Corre et al., 1997; Czyrak et al., 2002), suggesting receptor upregulation in response to low serotonergic tone. Some human studies are consistent with these findings, with corticosteroid treatment causing blunting of 5-HT_{1A} receptor-mediated responses, including the hypothermic and serum growth hormone responses to 5-HT_{1A} receptor agonists (Lesch et al., 1989; Young et al., 1994; Porter et al., 1998, 2002). However, overall, results are mixed and may depend on the type of corticosteroid used and the duration of exposure (Porter et al., 1999, 2002; Montgomery et al., 2001; Bhagwagar et al., 2003). Prior positron emission tomography (PET) imaging studies in humans have failed to detect an effect of acute administration of corticosteroids on 5-HT_{1A} receptor ligand binding (Montgomery et al., 2001; Bhagwagar et al., 2003). However, such studies did not examine stress-induced cortisol release, which may differ from pharmacologically induced cortisol effects and be abnormal in subgroups of patients.

To better understand the relationship between stress, HPA axis reactivity, depression, and 5-HT_{1A} receptor levels in the brain, we studied the relationship between the endogenous cortisol response to acute stress and 5-HT_{1A} receptor binding as measured by PET. We chose 2 different types of acute stressors: (1) the Trier Social Stress Task (TSST) (Kirschbaum et al., 1993), which is a psychological stress paradigm, and (2) arterial line placement prior to PET, a physical stressor that involves restraint and physical discomfort. Cortisol was measured in healthy volunteers and medication-free depressed subjects: (1) in a blood sample drawn just after arterial line placement, or (2) in saliva as part of the TSST. Given our finding of elevated [^{11}C]-WAY-100635 binding and HPA hyperactivity in MDD (Parsey et al., 2006a; Miller et al., 2009a; Parsey et al., 2010; Milak et al., 2018), we predicted a positive correlation between regional [^{11}C]-WAY-100635 binding and post-stress cortisol levels despite rodent studies that might predict an inverse relationship between the two. We predicted an inverse relationship between resting cortisol and regional [^{11}C]-WAY-100635 binding, as more severe depressive pathology is associated with higher 5-HT_{1A} binding on PET (Sullivan et al., 2015; Oquendo et al., 2016) and lower peripheral resting cortisol levels (Pfennig et al., 2005; Jokinen et al., 2010; McGirr et al., 2011; Melhem et al., 2016).

Methods

Participants

Seventy-three adult subjects with PET scans using [^{11}C]WAY-100635 were included in this analysis. Participants underwent 1

of 2 stressors: (1) arterial line placement before a PET scan (n = 34) or (2) the TSST (n = 39). Participants from stress paradigm (1) had cortisol assayed in a blood sample drawn immediately after their arterial line was placed, while participants of paradigm (2) had salivary cortisol assayed in samples collected at multiple time-points during the TSST. The PET data presented here were previously reported in published studies (Parsey et al., 2006a, 2006b, 2010; Miller et al., 2009b), but the cortisol data have never been published. Subject selection was based on the availability of usable [^{11}C]WAY-100635 brain binding data and either plasma cortisol or TSST saliva cortisol stress sample measurements.

Fourty-two depressed participants (25 female, 17 male) aged 18 to 62 years who met DSM-IV criteria for MDD in a current major depressive episode (as assessed by doctoral- or masters'-level psychologists and reviewed in a consensus conference of research psychologists and psychiatrists), a 17-item Hamilton Depression Rating Scale (HDRS) score REF ≥16, and capacity to provide informed consent were included in the analysis. Depression severity was assessed with the 24-item HDRS and the Beck Depression Inventory (BDI) REF. Thirty-one healthy volunteers (19 female, 12 male) aged 18 to 65 years with no history of DSM-IV Axis I or Axis II psychiatric disorders, no psychotropic medication exposure, and no family history of a mood disorder or schizophrenia were included. Neither group had significant medical illness, nor were they taking medications that may affect the serotonin system at the time of neuroimaging. For all subjects, the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 2012) as well as psychiatric and medical history, chart review, physical examination, routine blood tests, pregnancy test, urine toxicology, and electrocardiogram were performed to assess study eligibility. Exclusion criteria included presence of significant medical conditions, alcohol or other substance use disorder unless in complete remission for >6 months, dementia, neurological disease, head injury with loss of consciousness, pregnancy, first-degree family history of schizophrenia if subject was <33 years old (Sham et al., 1994), and >3 lifetime exposures to 3,4-methylenedioxymethamphetamine. For depressed participants specifically, exclusion criteria also included fluoxetine use within 6 weeks of PET scanning, or exposure to a 5-HT$_{1A}$ receptor agonist, such as buspirone, vortioxetine, vilazodone, or lysergic acid diethylamide, within 6 months of scanning. At the time of the scan all participants were unmedicated. Depressed subjects who were on ineffective antidepressant treatment at the time of evaluation underwent a medication washout and were drug-free for at least 2 weeks prior to neuroimaging. The Institutional Review Board of the New York State Psychiatric Institute approved the protocol, and all subjects provided informed written consent after an explanation of the study protocol and associated risks.

Radiochemistry and Input Function Measurement

Subjects were injected with [^{11}C]WAY-100635 for quantification of 5-HT$_{1A}$ binding. Details of radiotracer preparation have been previously described for [^{11}C]WAY-100635 (Parsey et al., 2000). A metabolite-corrected arterial input function was obtained and plasma free fraction (f_p) was assayed in triplicate (Parsey et al., 2006a).

Image Acquisition and Analysis

A T1-weighted magnetic resonance image (MRI) scan was acquired for each subject for registration with PET images using a 1.5-T Signa Advantage (General Electric Medical Systems, Milwaukee, WI) at a resolution of 1.5 × .9 × 1.0 mm.

PET images were acquired with an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN) as previously detailed (Parsey et al., 2000). Briefly, after a 15-minute transmission scan, an injection of [^{11}C]-WAY-100635 was administered over 30 seconds and then an emission scan of 110 minutes, consisting of 20 frames of increasing duration (3 × 20 seconds, 3 × 1 minute, 3 × 2 minutes, 2 × 5 minutes, 9 × 10 minutes), was obtained.

To correct for subject motion, each PET frame was registered to the eighth frame of the scan using the FMRIB linear image registration tool, version 5.0 (FMRIB Image Analysis Group, Oxford, UK). Brain regions of interest (ROIs) were chosen a priori based on areas of abundant [^{11}C]-WAY-100635 binding (Hall et al., 1997) that included raphe nuclei (RN), anterior cingulate, cingulate, dorsal prefrontal cortex, hippocampus, insula, medial prefrontal cortex, parietal cortex, parahippocampal gyrus, occipital cortex, orbital cortex, temporal cortex, and amygdala. Cerebellar white matter was used as a reference region. All ROIs except for RN were identified on each individual's T1-weighted MRI using a previously described automated algorithm (Milak et al., 2010). Due to their small size, RN were labeled using a standard space mask of the average location of the RN in 52 healthy subjects, which was created using [^{11}C]-WAY-100635 voxel binding maps as previously described (Delorenzo et al., 2013). MRI T1 images were transformed into standardized 3D space using Advanced Normalization Tools (7), and the reverse transform was applied to the RN mask.

PET images were co-registered to the MRI images using the FMRIB linear image registration tool, optimized as previously described (Delorenzo et al., 2009). The average activity measured over the voxels within each ROI over the specified time frames through the course of the acquisition generated time activity curves.

Outcome Measure Estimation

Distribution volumes (V_T) of [^{11}C]WAY-100635 were estimated for each ROI using kinetic analysis with an arterial input function and a 2-tissue compartment constrained model as previously described (Parsey et al., 2000). Time activity curves were fit with a 2-tissue compartment constrained model in which the K_1/k_2 ratio was constrained to that of the reference region (cerebellar white matter) for each ROI. BP_F was calculated as $(V_{T(ROI)} - V_{T(REF)})/f_p$, where $V_{T(ROI)}$ is the volume of distribution in a specific ROI, $V_{T(REF)}$ is the volume of distribution in the reference region, and f_p is the free fraction in plasma.

Cortisol Measurement

Blood cortisol was measured in blood samples drawn from 34 subjects immediately prior to PET scans and just after the arterial line was inserted. Given the diurnal variation of cortisol, all these blood samples were drawn within a 2-hour time window between 12 PM and 2 PM. In addition, we adjusted statistically for time of day when samples were drawn. Blood cortisol levels were ascertained by radioimmunoassay (Vecsei, 1979) after denaturation of the binding proteins by heat. Both blood and saliva cortisol levels were measured by immunoassay with antibodies for cortisol-3-O-carboxymethyloxime-BSA (MP Biochemicals). This was compared to cortisol standards (Sigma Chemical). Free and bound fractions were separated using anti-rabbit globulin serum and polyethylene glycol. All samples and standards were analyzed in duplicate.

Subjects who underwent the TSST, which took place between 2 PM and 6 PM, gave saliva samples approximately 10 minutes

prior to the start of the TSST ($Cort_{BL}$) and again at 15, 20, 30, and 40 minutes after completion of the TSST. Time of day was also statistically controlled for in this sample. Saliva samples were collected in the Sarstedt Salivette Synthetic Swab collection system (catalogue # 51.1534.500 Sarstedt, Newton, NC) and subsequently stored at –30°Celsius. Salivary cortisol values were log-transformed. Cortisol response during TSST for each subject was defined as the difference between the maximum (log-transformed) value after the baseline and the (log-transformed) baseline value. Baseline cortisol was also log-transformed.

TSST

Salivary cortisol response to social stress was measured in 39 subjects not overlapping with the subjects with blood cortisol measures during the PET scan: 29 MDD patients and 10 healthy volunteers. The TSST was administered as previously described (Melhem et al., 2016). In brief, subjects were asked to give a 5-minute personal introduction speech, followed by 9 minutes of speeded mental arithmetic, while being watched by 1 observer known to the subject and a staff member who was unknown to the subject.

Statistical Analysis

The associations between the cortisol responses to the 2 stressors and $5\text{-}HT_{1A}$ binding measured in our a priori ROIs were examined separately for each stressor, each analysis using linear mixed effects models with group (MDD vs control), cortisol, brain region, and their interactions as fixed effects and subject as a random effect. To correct a slight skew in the data, stabilize the variance across regions, and allow for estimates of proportional effects that persist across regions, the analysis was performed on log-transformed estimates of $5\text{-}HT_{1A}$. Observations were weighted inversely proportionally to squared standard errors that were calculated based on variation in PET data, plasma data, and metabolite data (Ogden and Tarpey, 2006). Because [^{11}C]WAY-100635 binding has been shown to be dependent on sex (Parsey et al., 2002) and age (Tauscher et al., 2001), these covariates were also included in the model initially, both as main effects and in the form of interaction with region, but interactions were dropped when not significant. When the 3-way interaction between region, group, and cortisol measure was significant, brain region-wise analyses were performed to test the association between cortisol, subject group, and binding. Covariates that were not significant in any region were dropped from region-wise analyses.

Given that cortisol has a diurnal cycle (Wüst et al., 2000), we adjusted blood cortisol measurements during the PET scan for time of day relative to 12:00 PM, even though the correlation between blood cortisol and time of day was not significant in this dataset ($P = .091$). This adjustment, performed to remove the time trend, was based on fitting a linear regression model to the cortisol data with time as the only predictor. Log-transformed baseline cortisol before the TSST was similarly adjusted, although the time trend was not statistically significant. Cortisol response during the TSST was adjusted by a time trend that differed between males and females, using an ANCOVA model with time, sex, and their interaction as predictors. Cortisol measurements were also compared between healthy volunteers and participants with MDD using an ANCOVA analysis, and, in MDD patients, were correlated with 24-item HDRS and BDI scores.

Results

Participant Characteristics

Demographic and clinical data are presented in Table 1 for all participants combined. Demographic and clinical data are presented for the separate samples of subjects with blood cortisol and those who underwent the TSST in Tables 2 and 3, respectively. Of 42 MDD participants, 29 had previous exposure to antidepressants and 16 had previously attempted suicide, indicating high illness burden. For those with previous antidepressant exposure, the mean time off antidepressants was 49 + 74 weeks (range 2–312 weeks).

Cortisol Stress Response in Healthy Volunteer and Major Depression Groups

There was no statistically significant difference in blood cortisol levels measured following radial artery catheter placement between control and MDD groups when covarying for age, sex, and blood sample time of day ($F = 3.70$; $df = 1,31$; $P = .064$). Salivary

Table 1. Demographic and Clinical Characteristics of Combined Study Sample

	Healthy Volunteers	MDD	All subjects	*P* value
	(n = 31)	(n = 42)	(n = 73)	
Age ± SD	35.3 ± 13.3	38.2 ± 12.6	37 ± 12.9	0.32
Hamilton Depression Rating Scale (24-item)	2.5 ± 3.2	18.9 ± 13.5		<.001
Beck Depression Inventory	1.9 ± 3.8	20.8 ± 16		<.001
Years of Education	15.7 ± 2.1	15.2 ± 2.5	15.45 ± 3.2	
	n (%)	n (%)	n (%)	*P* value
Female	17 (54.8)	25 (59.5)	42 (57.5)	.68
Tobacco users	3 (9.7)	5 (11.9)	8 (11)	.76
Prior exposure to antidepressants	N/A	29 (69)	29 (39.7)	
Suicide attempters	N/A	16 (38.1)	16 (21.9)	
Past alcohol abuse	N/A	7 (16.6)	7 (9.6)	
Comorbid anxiety disorder	N/A	19 (45.2)	19 (26)	
Race/ethnicity				.09
Asian	5 (16.1)	2 (4.8)	7 (8.3)	.10
African American	7 (22.6)	5 (11.9)	14 (16.7)	.22
Caucasian	15 (48.3)	31 (73.8)	55 (65.5)	.02
Hispanic	4 (12.9)	10 (23.8)	14 (16.7)	.2 2

Table 2. Demographic Information for Blood Cortisol Subjects Only

	Healthy Volunteers	MDD	All subjects	*P* value
	(n = 21)	(n = 13)	(n = 34)	
Age ± SD	34.3 ± 14.1	38.1 ± 13.6	35.8 ± 13.18	.44
Hamilton Depression Rating Scale (24-item)	1 ± 1.2	24.8 ± 8.2		<.001
Beck Depression Inventory	1.9 ± 3	28.5 ± 11.9		<.001
Years of education	15.9 ± 2.5	15.1 ± 3.3	15.6 ± 2.8	.41
	n (%)	n (%)	n (%)	*P* value
Female	11 (52.4)	10 (76.9)	21 (61.8)	.31
Tobacco users	2 (9.5)	1 (7.7)	3 (8.8)	.8
Prior exposure to antidepressants	N/A	9 (69.2)	9 (26.5)	
Suicide attempters	N/A	8 (61.5)	8 (23.5)	
Past alcohol abuse	N/A	4 (30.7)	4 (11.8)	
Comorbid anxiety disorder	N/A	6 (46.2)	6 (17.6)	
Race/ethnicity				.88
Asian	4 (19)	1 (7.7)	5 (14.7)	.36
African American	5 (23.8)	1 (7.7)	6 (17.6)	.23
Caucasian	9 (42.9)	7 (53.8)	16 (47.1)	.53
Hispanic	3 (14.3)	4 (30.8)	7 (20.6)	.24

Table 3. Demographics of TSST Subjects

	Healthy Volunteers	MDD	All subjects	*P* value
	(n = 10)	(n = 29)	(n = 39)	
Age ± SD	38.2 ± 11.7	38.2 ± 12.6	38.8 ± 12	.85
Hamilton Depression Rating Scale (24-item)	2.6 ± 3	18.7 ± 10.7	14.6 ± 4.8	<.001
Beck Depression Inventory	1.9 ± 2.4	20.5 ± 10.8	15.6 ± 12.5	<.001
Years of education	15.2 ± 0.67	15.3 ± 2.1	15 ± 1.8	.94
	n (%)	n (%)	n (%)	*P* value
Female	6 (60)	15 (51.7)	21 (53.8)	.65
Tobacco users	1 (10)	2 6.9)	3 (7.7)	.75
Prior exposure to antidepressants	N/A	20 (69)		
Suicide attempters	N/A	8 (27.6)		
Past alcohol abuse	N/A	3 (10.3)		
Comorbid anxiety disorder	N/A	13 (44.8)		
Race/ethnicity				.64
Asian	1 (10)	1 (3.4)	2 (5.1)	.41
African American	2 (20)	4 (13.8)	6 (15.4)	.63
Caucasian	6 (60)	24 (82.8)	30 (76.9)	.14
Hispanic	1 (10)	6 (20.7)	7 (17.9)	.44

baseline cortisol and cortisol response during the TSST also did not differ significantly between control and MDD groups when covarying for age, sex, time of day, and the sex by time interaction ($F = 0.91$; $df = 1,33$; $P = .348$, baseline cortisol: $F = 2.51$; $df = 1,34$; $P = .122$).

Cortisol Stress Response and Depression Severity

Within the MDD sample, we did not find a relationship between PET scan blood cortisol levels and the BDI score ($F = 0.485$; $df = 1,8$; $P = .506$) or the 24-item HDRS score ($F = 0.455$; $df = 1,8$; $P = .518$), covarying for age, sex, and time of day in each case. The salivary cortisol response to TSST, covarying for age, sex, time of day, and their interaction, was not correlated with either 24-item HDRS score ($F = 0.56$; $df = 1,23$; $P = .462$) or BDI score ($F = 0.01$; $df = 1, 22$; $P = .936$).

Cortisol Stress Response and Brain [11C]WAY-100635 BP_F

[^{11}C]-WAY-100635 binding across the ROIs, selected a priori (Figure 1), was positively related with blood cortisol levels drawn immediately prior to the scan, after accounting for age, sex, and diagnosis ($F = 6.40$; $df = 1,29$; $P = .017$). The relationship between cortisol levels and [^{11}C]WAY100635 binding was homogenous across a priori brain regions, as the interaction term for brain region was not significant ($F = 0.67$; $df = 12,384$; $P = .777$). For region-wise results, see supplemental Table 1. There was no significant interaction between diagnosis and cortisol level on [^{11}C]WAY-100635 binding ($F = 3.77$; $df = 1,28$; $P = .062$).

Similarly, we found the time-adjusted salivary cortisol response during TSST was positively related with [^{11} C]WAY100635 binding after adjusting for sex and age (Figure 2; $F = 7.34$; $df = 1,34$; $P = .011$). This was also homogeneous across all a priori brain regions (region by cortisol response interaction: $F = 0.93$; $df = 11,374$; $P = .516$). For region-wise results, please see supplemental Table 2. There was no significant interaction between diagnosis and cortisol response on [^{11}C]WAY-100635 binding ($F = 0.21$; $df = 1,33$; $P = .649$). There was also no main effect of diagnosis after removing the interaction ($F = 0.01$; $df = 1,34$; $P = .988$). There was a significant age by region interaction ($F = 2.31$; $df = 11,396$; $P = .009$) and a significant sex by region interaction

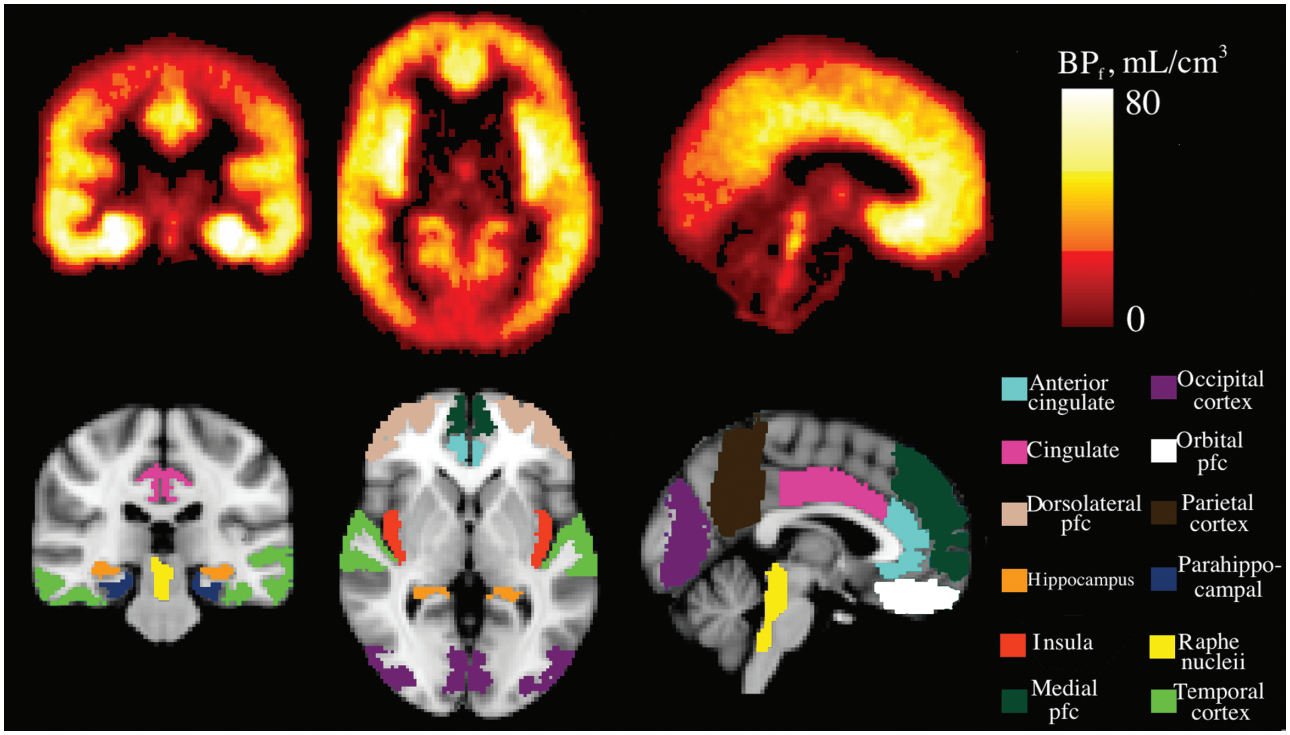

Figure 1. The post-stress cortisol is positively correlated with serotonin 1A (5-HT_{1A}) receptor ligand binding across multiple brain regions. Top row shows [^{11}C]WAY-100635 BP_f averaged across subjects. Bottom row highlights corresponding anatomical regions of interest (ROIs) for which a significant positive correlation between [^{11}C]WAY-100635 binding and blood cortisol levels was observed, including the raphe nuclei, cingulate cortex, dorsal prefrontal cortex, hippocampus, insula, medial prefrontal cortex, parietal cortex, parahippocampal gyrus, occipital cortex, orbital cortex, and temporal cortex. Analyses were corrected for multiple comparisons using a threshold of $P < .001$.

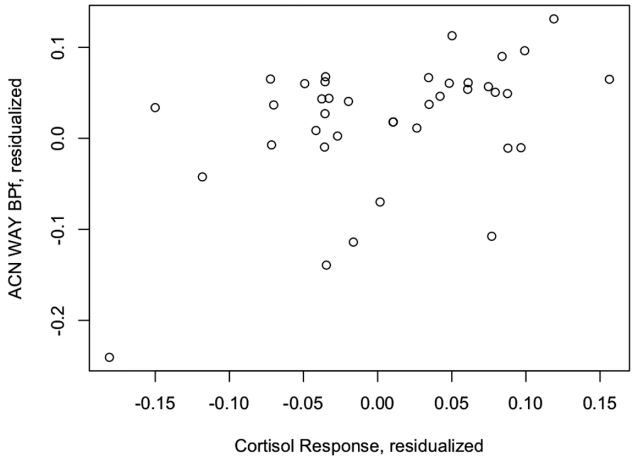

Figure 2. Cortisol response during the Trier Social Stress Task (TSST) is positively correlated with [^{11}C]WAY-100635 BP_F. Shown here are the residual values for [^{11}C]WAY-100635 BP_F in a single region of interest (ROI), the anterior cingulate (ACN), and corresponding residual values for cortisol response after controlling for age, sex, and diagnosis.

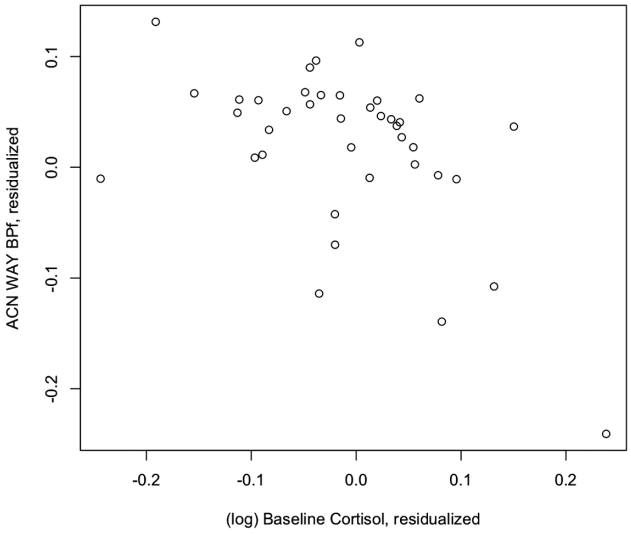

Figure 3. Baseline cortisol response during the Trier Social Stress Task (TSST) is inversely correlated with [^{11}C]WAY-100635 BP_F. Shown here are the residual values for [^{11}C]WAY-100635 BP_F in a single region of interest (ROI), the anterior cingulate (ACN), and corresponding residual values for cortisol response after controlling for age, sex, and diagnosis.

($F = 3.01$; $df = 11,396$; $P = .001$), indicating differential effects of these demographic variables on binding across brain regions.

Baseline Cortisol and Brain [^{11}C]WAY-100635 BP_F

The association between baseline salivary cortisol measured before the TSST and [^{11}C]WAY100635 binding differed by region and diagnostic group (baseline cortisol by region by group interaction: $F = 2.41$; $df = 11,374$; $P = .007$). To interpret the interaction, we ran posthoc region-wise analyses adjusted for age, sex, and diagnostic group. The effects of the interaction terms of diagnostic group with baseline cortisol on [^{11}C]WAY100635 binding were not significant in any of the region-specific models and were removed. The main effect of diagnosis was not significant in any region. Baseline cortisol and [^{11}C]WAY100635 binding

were negatively correlated in all regions (Figure 3; see supplemental Table 3 for coefficients by ROI) except in the RN (b = –0.18; SE = 0.11; t = –1.71; $P = .098$).

Discussion

Here we show a positive correlation between cortisol levels after two different types of stressors and 5-HT_{1A} receptor binding on PET using [^{11}C]WAY-100635. This effect was observed across multiple brain regions. The fact that this observation held under 2 different stress paradigms speaks to the strength of this relationship. Conversely, a negative correlation was found between baseline salivary cortisol and [^{11}C]WAY-100635 binding in all a priori regions, except RN. We previously reported that stress-responsive disorders like MDD and PTSD are associated with higher 5-HT_{1A} binding (Parsey et al., 2006a, 2010; Sullivan et al., 2013), although other studies have found no difference or the opposite (Yates and Ferrier, 1990; Lowther et al., 1997; Sargent et al., 2000; Bonne et al., 2005; Sullivan et al., 2015; Mann et al., 2017). In this study, the relationship of 5-HT_{1A} binding to post-stress cortisol in blood and salivary samples was independent of diagnosis, indicating a mechanism that operates comparably in healthy volunteers and in patients with mood disorders. Consistent with this observation, within the MDD group the severity of current major depression was not correlated with either 5-HT_{1A} binding or post-stress cortisol in either blood or salivary measures. Higher brain 5-HT_{1A} receptor ligand binding is a biological trait observed in medication-free major depression during acute depression and during remission (Parsey et al., 2006a, 2006b; Miller et al., 2009b; Parsey et al., 2010) and is transmitted in families (Milak et al., 2018). What remains to be determined is whether there is a causal link between higher 5-HT_{1A} binding and HPA axis overactivity or responsivity in depression.

A negative correlation was found between baseline salivary cortisol and [^{11}C]WAY-100635 binding in all a priori regions, except RN. This is consistent with previous data in depression and social anxiety disorder (Lanzenberger et al., 2010). That baseline cortisol has a weaker or no correlation with 5-HT_{1A} binding in RN may be explained by RN being a small structure with noisier quantification. Moreover, because of this small size, RN is susceptible to partial volume effects and underestimation of binding. Alternatively, presynaptic 5-HT_{1A} receptors (autoreceptors) in RN may be functionally distinct from post-synaptic 5-HT_{1A} receptors such that expression of the latter may be more strongly modulated by baseline cortisol levels. The latter explanation is consistent with the observation that adrenalectomy has no effect on 5-HT_{1A} receptor binding in RN (Le Corre et al., 1997; van Gaalen et al., 2002). Furthermore, animal work has shown that different types of stressors can lead to increased serotonin secretion within parts of the RN, which would then act on 5-HT_{1A} autoreceptors and differentially affect serotonin release in terminal fields (Adell et al., 1997, 2002). This may also explain why [^{11}C]WAY-100635 binding differs between the RN and terminal fields.

Previous animal work demonstrating an inverse relationship between cortisol levels and 5-HT_{1A} receptor expression in cortical and hippocampal regions are consistent with our findings with basal salivary cortisol levels (Chalmers et al., 1993; Meijer and de Kloet, 1994; Flügge, 1995; Zhong and Ciaranello, 1995; Le Corre et al., 1997; Czyrak et al., 2002; Iyo et al., 2009). It is thought that cortisol-dependent transcriptional repression of 5-HT_{1A} requires coactivation of both the glucocorticoid and mineralocorticoid receptors (Meijer et al., 2000; Ou et al., 2001). However, it is important to note that in addition to possible species differences, the animal experiments differ markedly from our paradigm in that they were performed either in animals in the context of adrenalectomy, chronic steroid treatment, or chronic stress paradigms, or in cell culture. We did not measure baseline cortisol in our blood cortisol samples, which were taken after arterial line insertion prior to PET scan. Therefore, the post-stress blood cortisol measures represent a combination of the resting cortisol levels and the response to the stressor, and they cannot be disambiguated. We considered the potential contribution of circadian fluctuations in cortisol level by adjusting TSST cortisol level for time of day in the model.

HPA axis dysfunction has been linked to depression and suicide in many previous studies. Postmortem data from suicide completers shows that they tend to have heavier adrenal glands, higher tissue levels of CRF, which indicates oversecretion of CRF, and lower expression of CRF receptors in prefrontal cortex (Nemeroff et al., 1988; Arató et al., 1989; Szigethy et al., 1994). A subset of patients with depression, generally those with more severe illness, also demonstrate nonsuppression on dexamethasone suppression testing and are at higher risk of suicide (Caroff et al., 1983; Dratcu and Calil, 1989; Coryell and Schlesser, 2001; van Heeringen, 2003; Yerevanian et al., 2004; Pfennig et al., 2005; Kunugi et al., 2006). HPA axis dysfunction is also linked directly to serotonergic dysfunction and specifically to changes in 5-HT_{1A} receptor levels. CRF directly affects the dorsal raphe, modulating serotonergic tone in the prefrontal cortex and nucleus accumbens (Lowry et al., 2000; Forster et al., 2008; Lukkes et al., 2008; Quadros et al., 2014). Several studies in animal models show that cortisol reduces 5-HT_{1A} receptor expression in the hippocampus (Martire et al., 1989; Chalmers et al., 1993; Kuroda et al., 1994; Meijer and de Kloet, 1994; Zhong and Ciaranello, 1995). Our data add to this literature by demonstrating a functional relationship between 5-HT_{1A} receptor binding levels and cortisol levels in response to an acute stressor in human subjects.

The study had several limitations. There were modest differences in racial/ethnic composition between our healthy volunteers and MDD group. Subjects in this study were a convenience sample, included on the grounds of having undergone [^{11}C]WAY-100635 imaging and having either a blood or salivary measure of cortisol available. We cannot determine whether these serotonin system relationships to baseline and post-stress cortisol levels will extend to more severe MDD, which is characterized by cortisol hypersecretion and dexamethasone resistance. However, we saw no relationship between MDD severity across the range that was present in our sample and cortisol measures. Finally, this cross-sectional study cannot demonstrate causal relationships. This would be more feasible to study in mouse models where pharmacological and genetic manipulations of HPA axis responsiveness are possible and the time frame to determine the relationship of developmental effects on adult phenotypes much shorter. To more fully characterize the relationship of depression status to HPA axis reactivity, a longitudinal study with repeated measurements in individual subjects during and between episodes of major depression would be required and would complement mouse developmental studies.

In summary, despite limitations, we found a positive correlation between 5-HT_{1A} receptor and post-stress cortisol levels, independent of diagnosis. This suggests an underlying mechanism that links 5-HT_{1A} receptor overexpression with HPA axis feedback dysfunction. Conversely, binding and resting cortisol are negatively correlated as reported in several rodent studies and likely involve different mechanisms including the mineralocorticoid receptor.

Such mechanisms are a combination of genetic vulnerability and environmental risk factors, such as early-life stress, which leads to epigenetic changes generating this biological phenotype.

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