

REGULAR RESEARCH ARTICLE

Brain 5-HT_{1A} Receptor PET Binding, Cortisol Responses to Stress, and the Familial Transmission of Suicidal Behavior

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Abstract

Background. The serotonin 1A (5-HT_{1A}) receptor has been implicated in depression and suicidal behavior. Lower resting cortisol levels are associated with higher 5-HT_{1A} receptor binding, and both differentiate suicide attempters with depression. However, it is not clear whether 5-HT_{1A} receptor binding and cortisol responses to stress are related to familial risk and resilience for suicidal behavior.

Methods. [¹¹C]CUMI-101 positron emission tomography imaging to quantify regional brain 5-HT_{1A} receptor binding was conducted in individuals considered to be at high risk for mood disorder or suicidal behavior on the basis of having a first- or second-degree relative(s) with an early onset mood disorder and history of suicidal behavior. These high-risk individuals were subdivided into the following groups: high risk resilient having no mood disorder or suicidal behavior (n=29); high risk with mood disorder and no suicidal behavior history (n=31); and high risk with mood disorder and suicidal behavior (n=25). Groups were compared with healthy volunteers without a family history of mood disorder or suicidal behavior (n=34). Participants underwent the Trier Social Stress Task (TSST). All participants were free from psychotropic medications at the time of the TSST and PET scanning.

Results. We observed no group differences in 5-HT_{1A} receptor binding considering all regions simultaneously, nor did we observe heterogeneity of the effect of group across regions. These results were similar across outcome measures (BP_{ND} for all participants and BP_p in a subset of the sample) and definitions of regions of interest (ROIs; standard or serotonin system-specific ROIs). We also found no group differences on TSST outcomes. Within the high risk with mood disorder and suicidal behavior group, lower BP_p binding ($\beta = -0.084$, SE = 0.038, $P = .048$) and higher cortisol reactivity to stress ($\beta = 9.25$, 95% CI

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Significance Statement

The serotonin 1A (5-HT_{1A}) receptor and HPA axis activity are implicated in suicidal behavior. However, it is not clear whether 5-HT_{1A} receptor binding and cortisol responses to stress are related to familial risk and resilience for suicidal behavior. Our results suggest that 5-HT_{1A} receptor PET binding was not linked to familial risk or resilience for suicidal behavior or mood disorder. However, lower 5-HT_{1A} receptor binding was associated with higher lethality of suicide attempt and higher cortisol reactivity to stress. Thus, altered 5-HT_{1A} receptor binding may represent a biomarker for more severe acute suicidal ideation and higher lethality suicidal behavior. These results provide further evidence linking HPA axis dysfunction to suicidal behavior.

[3.27,15.23], $P = .004$) were associated with higher lethality attempts. There were no significant relationships between 5-HT_{1A} binding and cortisol outcomes.

Conclusions. 5-HT_{1A} receptor binding in ROIs was not linked to familial risk or resilience protecting against suicidal behavior or mood disorder although it may be related to lethality of suicide attempt. Future studies are needed to better understand the biological mechanisms implicated in familial risk for suicidal behavior and how hypothalamic-pituitary-adrenal axis function influences such risk.

Keywords: 5-HT_{1A} Receptor, [¹¹C]CUMI-101 PET imaging, cortisol response to stress, familial risk, suicidal behavior

Introduction

Serotonergic system hypofunction is implicated in depression and suicidal behavior (Mann, 2013; Oquendo et al., 2014). The 5-HT_{1A} receptor, by regulating serotonin release via the autoreceptor or by signaling on serotonin target neurons throughout the brain, is involved in mood regulation, decision-making, suicidal behavior, aggression, memory, learning, and hippocampal neurogenesis (Radley and Jacobs, 2002; Ogren et al., 2008; Wang et al., 2016). Antidepressants downregulate serotonin (5-HT_{1A}) autoreceptors in the raphe nuclei (RN), the sole source of serotonin production and release in the brain, to augment serotonin neuron firing and release as part of their antidepressant mechanism of action (Haddjeri et al., 1998; Lan et al., 2013; Metts et al., 2019). In a meta-analysis of 10 positron emission tomography (PET) imaging studies, lower 5-HT_{1A} receptor binding in the mesiotemporal cortex was reported in depressed patients compared with healthy controls (Wang et al., 2016). However, other studies, which were not included in this meta-analysis, found elevated binding in depression during an acute episode and between episodes in remitted patients (Miller et al., 2009; Sullivan et al., 2015; Milak et al., 2018; Pillai et al., 2018). Higher binding was also observed in offspring of parents with major depression and was highest among those who went on to develop depression, indicating that this could be a familial trait (Milak et al., 2018). We also reported that in major depressive disorder (MDD), higher autoreceptor binding in the RN predicted higher suicidal ideation up to 1 year post scan and was positively associated with 5-HT_{1A} binding in prefrontal cortex and RN regions (Sullivan et al., 2015; Oquendo et al., 2016). It is not clear whether the discrepancy in 5-HT_{1A} receptor binding results in depression and suicidal behavior could be due to differences in a biological phenotype related to risk and/or resilience for mood disorders or suicidal behavior.

Hypothalamic-pituitary-adrenal (HPA) axis dysregulation is associated with alterations in the serotonergic system through many mechanisms, including a decrease in hippocampal 5-HT_{1A} receptor mediated function (Leonard, 2005; Mahar et al., 2014). Lower resting cortisol has been associated with higher 5-HT_{1A} receptor binding in all brain regions, except in the RN (Steinberg et al., 2019). Similarly, higher 5-HT_{1A} receptor binding in the mesiotemporal cortex was correlated with lower resting plasma cortisol (Nugent et al., 2013a). We previously reported

that suicide attempters who were offspring of parents with mood disorders had lower baseline cortisol and total cortisol output during the Trier Social Stress Task (TSST) compared with offspring who never attempted suicide and with healthy controls (Melhem et al., 2015; O'Connor et al., 2017). However, to our knowledge, there are no studies comparing cortisol response to stress and 5-HT_{1A} receptor binding in an offspring cohort of vulnerable and resilient individuals at high risk for major depression and suicidal behavior.

To address whether altered 5-HT_{1A} receptor binding is a vulnerability or resilience biological marker for major depression and/or suicidal behavior, we conducted a PET imaging study of 5-HT_{1A} receptor binding using [¹¹C]CUMI-101 in medication-free high-risk individuals having a first- or second-degree relative with both an early onset mood disorder and a history of suicidal behavior. All high-risk individuals were recruited when largely beyond the age of risk for mood and suicidal behavior, allowing us to assign participants to 1 of 3 groups—vulnerable to mood disorder, vulnerable to mood disorder and suicidal behavior, or resilient because they have not developed mood disorder or made a suicide attempt (SA)—and compare them with healthy controls. We also examined the relationship of 5-HT_{1A} receptor binding with cortisol responses to the TSST. Given our prior findings, we hypothesized higher 5-HT_{1A} receptor binding to be a familial biological phenotype associated with risk for suicidal behavior. We also hypothesized that higher 5-HT_{1A} receptor binding will be associated with the HPA axis biological phenotype previously associated with suicidal behavior and its familial transmission, that is, lower cortisol responses to stress.

METHODS

Sample

The sample consisted of medication-free high-risk individuals and healthy volunteers >25 years of age, an age by which mood disorder and suicidal behavior in our familial transmission studies are generally phenotypically expressed (Melhem et al., 2019). All high-risk participants had at least 1 first- or second-degree relative with early-onset mood disorder (MOOD) and a lifetime history of SA and were separated into vulnerable

and resilient groups based on their phenotype: high-risk individuals with mood disorders and a history of SA (HR-SA/MOOD, $n=25$), high-risk individuals with mood disorders only (HR-MOOD, $n=31$), and high-risk resilient individuals with no history of mood disorders or SA (HR-R, $n=29$). Healthy volunteers (HVs, $n=34$) had no personal or family history of psychiatric disorders and suicidal behavior. The sample was recruited at 2 sites, Pittsburgh and New York, in protocols approved by their respective institutional review boards, through outpatient clinics, clinician referrals, and institutional review board-approved advertisements; and these data were not previously reported on in any previous publications. Individuals with current alcohol and substance use disorders were excluded. At the New York site, individuals were included if they were unmedicated or taking medication that was ineffective after a trial of adequate dose and duration. Those individuals underwent medication taper followed by 3 weeks off medication prior to scanning. Participants across all groups were 67.2% females, mean age 38.0 years ($SD=10.1$), 61.9% White, and 12.7% Hispanic. Of these participants, 86.6% completed scanning procedures, 84.9% completed the TSST, and 73.9% completed both. Participants from the 2 sites did not differ on demographic and clinical characteristics except that those from the Pittsburgh site were more likely to have comorbid anxiety disorders (42.6% vs 20.5%, $\chi^2=3.63$, $df=1$, $P=.02$), had higher BMI (28.7 ± 6.2 vs 24.3 ± 4.5 , $t=4.14$, $P<.001$), and were less likely to be Hispanic (77.8% vs 95.3%, $\chi^2=0.29$, $df=1$, $P=.004$) (supplementary Table 1). Participants at the New York site were more likely to report history of childhood abuse (24.1% vs 7.7%, $\chi^2=6.17$, $df=1$, $P=.013$).

Assessment

Psychiatric diagnoses were assessed in all groups, including HVs, using the Structured Clinical Interview for DSM-5 disorders (SCID-5) (First et al., 2015). Suicidal behavior was assessed using the Columbia Suicide History Form and the Columbia Suicide Severity Rating Scale (C-SSRS), which assess lifetime history of attempts, the severity of ideation, and lethality of attempts (Posner et al., 2011). Lethality was assessed using Medical Lethality Scale (Beck et al., 1975) with a score ranging between 0 and 8, where 0 corresponds to no injury and 8 to death. The scale includes anchors for the medical damage caused by the attempt dependent on the method used. Self-reported symptoms were assessed using the Beck Depression Inventory (Beck et al., 1961) and the Beck Hopelessness Inventory (Beck et al., 1974). We also assessed aggression and impulsivity using the Brown-Goodwin Lifetime History of Aggression Scale (Brown and Goodwin, 1986) and the Barratt Impulsivity Scale (Barratt, 1965), respectively. Childhood abuse and neglect were assessed by self-report using the Childhood Trauma Questionnaire (Bernstein et al., 1994).

Trier Social Stress Task

We administered the TSST, a well-established protocol to induce psychological and endocrine indices of stress response (Kirschbaum et al., 1993), where participants gave a 5-minute personal introduction speech followed by 9 minutes of a speeded mental arithmetic task in the presence of an observer unknown to the participant. We did not apply the social evaluation component of the original TSST in which the observer provides negative feedback to reduce the degree of stress imposed on this high-risk population. In this modified TSST, we replaced criticism with indifference, and this still elicits cortisol responses indicating measurable stress effects (Melhem et al.,

2015). We collected salivary samples on arrival to our laboratory (Cort₋₃₀) and asked the participant to relax for 20 minutes, after which another sample was obtained 10 minutes before the procedure (Cort₋₁₀). We then collected samples at 15, 20, 30, and 40 minutes after completing the task. We administered the Profile of Mood States (McNair et al., 1992) at -10, 15, and 40 minutes to assess subjective mood. Cortisol levels were measured in ng/mL, and total cortisol output was measured using area under the curve with respect to ground (AUC_G) and cortisol reactivity using AUC with respect to increase (AUC_I) following the trapezoid method (Pruessner et al., 2003) using raw values. We also analyzed pre-task CORT₋₁₀. On examination of the distribution of Cort₋₁₀, AUC_G, and AUC_I, we found CORT₋₁₀ and AUC_G were not normally distributed and thus were transformed using a natural logarithmic transformation before analysis.

PET Imaging

PET procedures to quantify 5-HT_{1A} binding potential (BP_{ND} and BP_S) (Innis et al., 2007) were previously described in detail (Parsey et al., 2000; Ogden et al., 2007; DeLorenzo et al., 2009b; Milak et al., 2010; Zanderigo et al., 2013; Bartlett et al., 2019). To minimize motion, individual head molds were prepared out of polyurethane (Soule Medical, Tampa, FL, USA). PET scans were performed on a Siemens Biograph mCT PET-CT scanner in New York and Pittsburgh (Siemens/CTI, Knoxville, TN, USA). [¹¹C] CUMI-101, synthesized as described by the radioligand laboratories at each institution (Kumar et al., 2007; Milak et al., 2008, 2010), was injected as an i.v. bolus over 30 seconds. Emission data were collected in 3-D mode for 120 minutes using 21 frames of increasing duration (3×20 seconds, 3×1 minutes, 3×2 minutes, 2×5 minutes, 10×10 minutes).

Magnetic Resonance Imaging

The Pittsburgh and New York sites have standardized MRI protocols. MRI images were acquired on a GE 3 Tesla MR750 scanner at the New York site and on a Siemens 3T Trios scanner in Pittsburgh. A sagittal T1-weighted structural scan was acquired over the whole brain with the following parameters: repetition time, 6 milliseconds; echo time (TE), 2.4 milliseconds; flip angle, 8; 1-mm slice thickness (zero gap); 178 slices; and field of view, 256×256 mm. All images were reconstructed to a size of 256×256 with an isotropic resolution of 1 cubic mm. This T1-weighted structural image was used for identification of ROIs, gray-white matter segmentation, skull-stripping, and co-registration to PET images.

Image Analysis

Image analysis was performed in MATLAB (The Mathworks, Natick, MA, USA). Head motion was corrected by registering each PET frame to frame 8 using FLIRT version 5.0 (FMRIB linear image registration tool by the FMRIB Image Analysis Group, Oxford, UK). PET-to-MRI transformations were computed, after attenuation correction, filtered back projection reconstruction, and motion corrected using FLIRT with a mutual information cost function and 6 degrees of freedom. Eight different co-registration possibilities with varying source/target images and weighting masks were calculated as previously described (DeLorenzo et al., 2009a). PET frames were co-registered to the MRI for automated ROI labeling as previously described (DeLorenzo et al., 2009b; Milak et al., 2010). After transformation, results were verified by visual inspection. Automated labels for

13 brain regions and for the reference region and cerebellar gray matter were generated from an atlas previously created and applied by our group (DeLorenzo et al., 2009b; Milak et al., 2010). ROIs consisted of anterior cingulate cortex, amygdala, cingulate cortex, dorsolateral prefrontal cortex, hippocampus, insula, medial prefrontal cortex, occipital cortex, orbitofrontal cortex, parietal cortex, parahippocampal gyrus, raphe nuclei, and temporal cortex. Probabilistic ROIs are assigned to the MRI using an automated multi-label approach (Milak et al., 2010). To calculate the probability of an ROI label for a particular voxel, the transformed template ROIs assigned to that voxel were counted and divided by the total number of templates. Cortical regions were then gray matter masked following target subject segmentation.

Primary and Secondary PET Outcome Measures

Our primary outcome measure was the binding potential $BP_{ND} = f_{ND} B_{avail} / K_D$, where f_{ND} is the fraction of free tracer in the non-displaceable tissue compartment, B_{avail} is the concentration of 5-HT_{1A} receptor that is available to bind to the tracer, and K_D is the tracer equilibrium dissociation constant. BP_{ND} was used because its quantification does not require blood sampling. On an ROI level, the optimal modeling method for obtaining BP_{ND} , using a reference region approach, has been determined by our group (Milak et al., 2010; Zanderigo et al., 2013) to be the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996). We therefore quantified BP_{ND} at the regional level as follows. In each participant, the tracer average time activity curve (TAC) (across all voxels) within each of the ROIs and in the gray matter cerebellum was extracted. Then, the gray matter cerebellum TAC was used in each participant as the reference tissue for the SRTM, which is implemented according to original publication in our in-house MATLAB-based software to perform kinetic modeling of PET data called *BrainFit*. SRTM provides an estimate of BP_{ND} in each region. SEs associated with BP_{ND} estimates were obtained according to bootstrapping procedures described elsewhere (Ogden and Tarpey, 2006; Zanderigo et al., 2013). In a subset of participants for whom 1 venous blood sample was acquired during PET imaging at 60 minutes after tracer injection, we also estimated the binding potential BP_p as previously described (Ogden, 2003; Ogden et al., 2010; Bartlett et al., 2019). Briefly, regional total distribution volume values of [¹¹C]CUMI-101 were first calculated in each participant and in each of the ROIs using Likelihood Estimation in Graphical Analysis (Ogden, 2003) and a simultaneously estimated input function with the single venous blood sample as the anchor, as previously validated (Ogden et al., 2010; Bartlett et al., 2019). Likelihood Estimation in Graphical Analysis was applied to each TACs using, at each data point in time, a weight equal to the square root of the corresponding acquisition frame duration, and a $t^* = 45$ minutes post injection, as previously validated (Milak et al., 2010). TACs were not corrected for vascular contribution, as multiple measures of radiotracer total radioactivity in whole blood were not available. BP_p was then calculated in each ROI as $BP_p = V_{T,ROI} - V_{T,CER}$, with $V_{T,CER}$ the volume of distribution estimated in the reference region, indicating the tracer's non-displaceable binding. BP_p is an estimate of $f_p B_{avail} / K_D$, where B_{avail} is the number of receptors available to bind to the radiotracer, $1/K_D$ is the affinity of the radiotracer for the receptor, and f_p is the tracer free fraction in plasma.

For estimation of both BP_{ND} and BP_p in a new serotonin-specific atlas (NRU 5-HT Atlas) (Beliveau et al., 2017, 2020) (see

below), voxel-level parametric maps of both binding potentials were obtained as follows. BP_{ND} was quantified at the voxel level using the reference tissue version of the likelihood estimation in graphical analysis (Zanderigo et al., 2013), which at the voxel level proved to be the method providing the closest estimation of BP_{ND} to values obtained based on arterial blood for [¹¹C]CUMI101 (Zanderigo et al., 2013). Voxel-wise V_T maps were computed for those participants with an associated single venous sample by using the input function simultaneously estimated at the ROI level (as described above) as the input to the Empirical Bayesian Estimation in Graphical Analysis (Zanderigo et al., 2010). Corresponding BP_p maps were then obtained by calculating in each voxel $BP_p = V_T - V_{T,ref}$ with V_T the tracer total distribution volume estimated by Empirical Bayesian Estimation in Graphical Analysis in each voxel and $V_{T,ref}$ the average V_T values among the voxels within the reference region (gray matter cerebellum).

PET Quantified in a Serotonin-Specific Atlas

In addition to quantifying BP_{ND} and BP_p in our group's standardly considered anatomic ROIs, we also quantified BP_{ND} and BP_p in a new serotonin-specific atlas (NRU 5-HT Atlas) (Beliveau et al., 2017, 2020). This atlas was previously generated by clustering voxel-wise PET binding maps from many participants across PET tracers targeting the serotonin transporter and 1A, 1B, 2A, and 4 receptors (Beliveau et al., 2020). It includes 20 stable regions (10 in each hemisphere) that represent unique signatures of serotonin signaling across the different receptors. NRU 5-HT Atlas FreeSurfer overlays were downloaded from <https://xtra.nru.dk/FS5ht-atlas/>. Each participant's T1-weighted MRI was first run through FreeSurfer 7.1.1 (<http://surfer.nmr.mgh.harvard.edu/>) (Fischl et al., 1999). For this analysis, grey-matter masked, voxel-wise BP_{ND} and BP_p maps quantified as described above were generated and coregistered to the MRI so that the maps could be resampled to the FreeSurfer *fsaverage* surface. Average BP_{ND} and BP_p estimates were then extracted from each of the 20 serotonin system-specific regions (left and right) by averaging all positive BP_{ND} or BP_p values within each region.

Statistical Analyses

We compared the 4 groups (HR-SA/MOOD, HR-MOOD, HR-R, and HV) on demographic and clinical characteristics, and on TSST measures using ANOVA with post hoc comparisons corrected for multiple comparisons. We examined group differences on TSST outcomes using linear regression with each of $CORT_{-10}$, AUC_c , and AUC_i as the dependent variable and group as the independent variable and controlled for covariates significantly associated with each outcome. Group was used as a dummy variable with HV as the reference group. We also explored interactions between group and significant covariates. We used a Bonferroni correction to correct for the 3 TSST outcomes and used $\alpha = 0.05/3 = 0.017$ as the threshold for significance. For PET outcomes, we used a mixed-effects model approach at the ROI level with region and group as fixed effects and with participant as a random effect to analyze BP_{ND} after weighting the estimated binding values in the models according to their estimated variances (Ogden and Tarpey, 2006). We used the *LmerTest* package in R with the Satterthwaite's method to compute the denominator degrees of freedom and F statistics. To prevent any individual data points from having an undue influence on the mean due

to chance underestimation of standard errors, weights were winsorized, with the largest weights set to the weight at the 70th percentile within each region (Potter, 1990). We controlled for sex, age, race, and site. Additional covariates were included the model as fixed effects. We selected the most parsimonious model as the final model, which included group as a dummy coded variable and covariates that were significant. To examine whether there were group differences in specific regions of the brain, we examined group by region interaction in these models. Before we applied mixed-effects models, data were log transformed to stabilize the variance and because our principal hypothesis of a difference between groups involved proportional differences across ROIs, which we and others have previously applied (Miller et al., 2009; Sullivan et al., 2009; Parsey et al., 2010). Finally, we examined the relationships between BP_{ND} from various ROIs with each of $Cort_{-10}$, AUC_G , and AUC_I . Exploratory analyses were also conducted on the subset of individuals with BP_p to allow comparisons to

BP_{ND} and extant literature. Similar analyses were conducted for standard ROIs and serotonin-specific ROIs.

RESULTS

Characteristics of the Study Sample

The HR-SA/MOOD, HR-MOOD, HR-R, and HV groups did not differ in terms of sex, age, race, or ethnicity (Table 1). The HR-SA/MOOD and HR-MOOD groups were more likely to have a lifetime history of comorbid anxiety disorders compared with the HR-R (72.7% vs 57.1% vs 14.3%, respectively; Fisher's Exact Test [FET], $P < .001$). Similar results were obtained for group differences on current anxiety disorders. The HR-SA/MOOD group was more likely to have a lifetime history of comorbid alcohol and substance use disorders compared with the HR-R (59.1% vs 17.9%; FET, $P = .004$). The HR-SA/MOOD group was also more likely than the HR-MOOD group to be in a current depressive episode (59.1%

Table 1. Demographic and Clinical Characteristics, and Cortisol Outcome Measures of the Study Sample

	HV n=34	HR-R n=29	HR-SA/MOOD n=25	HR-MOOD n=31	Test(df)	P
Sex, % (n) Males	35.3 (12)	34.5 (10)	32.0 (8)	29.0 (9)	$\chi^2(3) = 0.34$.952
Age, (mean \pm SD)	41.26 \pm 9.83	36.83 \pm 11.06	36.21 \pm 10.06	37.03 \pm 9.21	F(3,114) = 1.68	.175
Ethnicity, % (n) non-Hispanic	91.2 (31)	93.1 (27)	70.8 (17)	90.3 (28)	7.46	.059
Race, % (n) white	61.8 (21)	58.6 (17)	58.3 (14)	67.7 (21)	0.71	.871
Site, % (n) NY	50.0 (17)	41.4 (12)	48.0 (12)	41.9 (13)	0.70	.874
BMI, (mean \pm SD)	25.27 \pm 4.84	27.47 \pm 5.87	28.56 \pm 7.66	27.01 \pm 6.68	F(3,114) = 1.42	.239
Smoking, % (n)	5.9 (2)	17.2 (5)	28.0 (7)	29.0 (9)	FET	.053
Lifetime history of psychiatric disorders						
Major depressive disorder, % (n)	-	-	77.3 (17) ^b	89.3 (25) ^b	$\chi^2(1) = 1.32$.25
Bipolar disorder, % (n)	-	-	18.2 (4) ^b	3.6 (1) ^b	FET	.16
Mood disorders, % (n)	-	-	95.5 (21) ^b	92.9 (26) ^b	$\chi^2(1) = 0.15$.70
Anxiety disorders, % (n)	-	14.3 (4) ^a	72.7 (16) ^b	57.1 (16) ^b	FET	<.001
Posttraumatic stress disorder (PTSD), % (n)	-	-	31.8 (7) ^a	7.1 (2) ^a	FET	.49
Alcohol and substance use disorders, % (n)	-	17.9 (5) ^a	59.1 (13) ^b	28.6 (8) ^{ab}	FET	.03
Current psychiatric disorders						
Major depressive disorder, % (n)	-	-	59.1 (13) ^b	28.6 (8) ^c	$\chi^2(1) = 4.71$.03
Bipolar disorder, % (n)	-	-	9.1 (2)	0.0 (0)	FET	.08
Mood disorders, % (n)	-	-	68.2 (15) ^b	28.6 (8) ^c	$\chi^2(1) = 7.78$.01
Anxiety disorders, % (n)	-	10.7 (3) ^a	59.1 (13) ^b	46.4 (13) ^{ab}	FET	<.001
Posttraumatic stress disorder (PTSD), % (n)	-	-	9.1 (2)	7.1 (2)	FET	>.999
Alcohol and substance use disorders, % (n)	-	0.0 (0)	4.5 (1)	0.0 (0)	FET	.28
Self-reported symptoms						
Depression symptoms, (mean \pm SD)	1.53 (2.26) ^a	2.03 (2.96) ^b	19.20 (11.55) ^c	0.35 (9.28) ^d	F(3,115) = 34.05	<.001
Hopelessness, (mean \pm SD)	1.44 \pm 1.65 ^a	1.52 \pm 2.44 ^a	8.84 \pm 6.35 ^b	5.00 \pm 6.24 ^c	F(3,115) = 16.29	<.001
Aggression, (mean \pm SD)	12.03 (2.56) ^a	13.89 (3.75) ^{ac}	20.60 (5.38) ^b	16.37 (5.46) ^c	F(3,108) = 18.12	<.001
Impulsivity, (mean \pm SD)	33.83 (11.91) ^a	38.04 (20.91) ^a	55.70 (18.55) ^b	38.96 (15.68) ^a	F(3,93) = 7.37	<.001
Family history of suicide attempt in 1 st degree relatives, % (n)	-	78.6 (22)	71.4 (15)	82.8 (24)	FET	.62
Psychotropic medications, % (n)	-	6.9 (2) ^a	80.0 (20) ^b	64.5 (20) ^b	FET	<.001
Childhood abuse, % (n)	2.9 (1) ^a	6.9 (2) ^a	40.0 (10) ^b	16.1 (5) ^{ab}	FET	.001
TSST measures						
Baseline cortisol ($Cort_{-10}$), (mean \pm SD)	0.97 \pm 0.57	0.77 \pm 0.44	0.88 \pm 0.51	0.87 \pm 0.55	F(3,106) = 0.71	.549
Total cortisol output (AUC_G), (mean \pm SD)	68.4 \pm 32.9	55.2 \pm 33.6	61.9 \pm 37.6	60.5 \pm 33.8	F(3,97) = 0.68	.568
Cortisol reactivity (AUC_I), (mean \pm SD)	10.5 \pm 30.5	12.5 \pm 34.3	5.54 \pm 27.3	9.55 \pm 27.5	F(3,97) = 0.21	.891
POMS before TSST (-10 mins), (mean \pm SD)	0.79 \pm 14.09 ^{ab}	-7.43 \pm 12.31 ^a	31.54 \pm 36.96 ^c	11.07 \pm 22.47 ^b	F(3,111) = 14.55	<.001
POMS after TSST (15 mins), (mean \pm SD)	9.00 \pm 19.74 ^a	3.86 \pm 22.95 ^a	58.39 \pm 43.18 ^b	20.18 \pm 21.93 ^a	F(3,105) = 19.75	<.001
POMS after TSST (40 mins), (mean \pm SD)	1.13 \pm 13.75 ^{ab}	-6.54 \pm 13.46 ^a	37.26 \pm 34.00 ^c	10.29 \pm 24.69 ^b	F(3,105) = 17.86	<.001

Abbreviations: HV, healthy volunteers without a family history of mood disorder or suicidal behavior; HR-R, high-risk resilient with no mood disorder or suicidal behavior; HR-SA/MOOD, high-risk with mood disorder and suicidal behavior; HR-MOOD, high-risk with mood disorders and no suicidal behavior.

Superscripts are used for posthoc comparisons. POMS is the Profile of Mood States.

vs 28.6%, respectively; FET, $P < .001$). The HR-SA/MOOD had more severe current self-reported symptoms of depression and hopelessness, and impulsivity and aggression compared with all other groups. These clinical differences are all reported clinical correlates of suicidal behavior (Mann et al., 1999; Melhem et al., 2007; Brent et al., 2015). Finally, the HR-SA/MOOD reported higher rates of childhood physical or sexual abuse compared with all other groups.

TSST Outcomes by Group

Supplementary Tables 1 and 2 and supplementary Figure 1 present site differences and the relationships of all TSST outcomes with demographic and clinical characteristics, respectively. Controlling for site, and for covariates significantly associated with each of the cortisol measures (supplementary Table 2), there were no differences between the 4 groups (Table 2). Within HR-SA/MOOD, the group with a history of SA, we found higher AUC_i (cortisol reactivity to stress) to be associated with maximum lethality of attempt ($\beta = 9.25$, 95% CI [3.27, 15.23], $P = .004$). CORT₋₁₀, AUC_G, and AUC_i were not associated with self-reported severity of current depression or hopelessness and were not correlated with lifetime impulsivity and aggression across all groups. CORT₋₁₀ was correlated with AUC_G ($r = 0.63$, $P < .001$) and negatively correlated with AUC_i ($r = -0.28$, $P = .005$). AUC_G and AUC_i were also correlated ($r = 0.5$, $P < .001$).

PET Outcomes Using Standard ROIs by Group

Using mixed models and controlling for sex, age, race, and site, we found no overall group difference in 5-HT_{1A} BP_{ND} ($F[3,92.9] = 1.94$, $P = .129$). Our results showed small effect sizes for HR-SA/MOOD (Cohen $d = 0.226$) and HR-MOOD (Cohen $d = 0.204$) compared with HV but not for HR-R (Cohen $d = -0.084$) (Table 3; supplementary Figure 2). With the same approach, we observed no significant group by region interaction ($F[39, 612.5] = 0.895$, $P = .654$). There was no significant relationship between BP_{ND} binding and maximum lethality of attempt in the HR-SA/MOOD group controlling for site ($\beta = -0.036$, $SE = 0.031$, $P = .25$). We also found no relationships between PET binding and CORT_{-OR}

($\beta = -0.025$, $SE = 0.032$, $P = .440$), AUC_G ($\beta = -3.3 \times 10^{-4}$, $SE = 5.3 \times 10^{-4}$, $P = .536$), or AUC_i ($\beta = -2.6 \times 10^{-4}$, $SE = 6.3 \times 10^{-4}$, $P = .686$).

Similar results were found using the subset of individuals for whom BP_p could be estimated. Controlling for sex, age, race, and site, we found no group differences in BP_p ($F[3,41] = 1.0$, $P = .402$) (supplementary Table 3). Similarly, there was no significant group by region interaction for BP_p ($F[39, 273.14] = 1.05$, $P = .391$). However, we found a significant relationship between BP_p binding and maximum lethality of attempt among HR-SA/MOOD while controlling for site whereby lower BP_p binding was associated with higher lethality ($\beta = -0.084$, $SE = 0.038$, $P = .048$). Similar to BP_{ND}, there were no significant relationships between BP_p and TSST outcomes of CORT₋₁₀ ($\beta = -0.034$, $SE = 0.082$, $P = .678$), AUC_G ($\beta = -0.0015$, $SE = 0.001$, $P = .26$), and AUC_i ($\beta = -7.7 \times 10^{-4}$, $SE = 0.0014$, $P = .593$).

PET Outcomes Using Serotonin-Based ROIs by Group

We conducted additional analyses examining binding within ROIs defined by a serotonin-specific atlas and observed similar results. There were significant BP_{ND}-binding differences by region within the atlas ($F[19, 1938] = 1919.3$, $P = 2 \times 10^{-6}$), but no group ($F[3,99] = 1.36$, $P = .259$) or group by region interaction ($F[60, 981.4] = 1.28$, $P = .075$) effects were observed. Considering all ROIs simultaneously, BP_{ND} was not associated with CORT₋₁₀ ($\beta = -0.03$, $SE = 0.03$, $P = .316$), AUC_G ($\beta = -4.1 \times 10^{-4}$, $SE = 4.9 \times 10^{-4}$, $P = .409$), or AUC_i ($\beta = 3.5 \times 10^{-5}$, $SE = 5.8 \times 10^{-4}$, $P = .953$).

For BP_p, there were binding differences by region within the atlas ($F[19, 912] = 514.5$, $P = 2.2 \times 10^{-6}$), but no group ($F[3,43] = 0.44$, $P = .726$) or group by region interaction ($F[60, 430.6] = 1.0$, $P = .472$) effects were observed. Considering all ROIs simultaneously, BP_p was also not associated with CORT₋₁₀ ($\beta = -0.117$, $SE = 0.083$, $P = .166$), AUC_G ($\beta = -0.0015$, $SE = 0.0014$, $P = .284$), or AUC_i ($\beta = 3.9 \times 10^{-4}$, $SE = 0.0015$, $P = .797$).

Discussion

We found no significant differences between groups defined based on risk and resilience for the familial transmission of either mood disorder or suicidal behavior with regard to brain 5-HT_{1A} receptor binding and cortisol responses to the TSST and no

Table 2. Relationship of TSST Outcomes by Group Controlling for Covariates Significantly Associated with Each TSST Outcome

	β	95% CI	P	Cohen's d
CORT ₋₁₀				
HR-SA/MOOD	-0.12	(-0.40, 0.16)	.403	-0.080
HR-MOOD	-0.07	(-0.33, 0.18)	.566	-0.055
HR-R	0.16	(-0.42, 0.10)	.232	-0.115
Site, NY	0.19	(-0.01, 0.38)	.065	0.178
Ethnicity, non-Hispanic	-0.39	(-0.71, -0.07)	.019	-0.228
AUC _G				
HR-SA/MOOD	-6.73	(-26.0, 12.6)	.491	-0.069
HR-MOOD	-6.06	(-23.9, 11.8)	.502	-0.067
HR-R	-11.8	(-29.9, 6.4)	.199	-0.129
Site, NY	16.3	(2.84, 29.7)	.018	0.239
AUC _i				
HR-SA/MOOD	3.53	(-18.0, 16.0)	.658	0.044
HR-MOOD	-1.03	(-13.7, 18.2)	.905	-0.012
HR-R	2.28	(-12.3, 19.3)	.778	0.028
Site, NY	-5.54	(-17.6, 6.55)	.365	-0.091
Race, white	13.8	(1.44, 26.1)	.029	0.221
Smoking	-13.3	(-29.9, 3.31)	.115	-0.158

Abbreviations: HV, healthy volunteers without a family history of mood disorder or suicidal behavior; HR-R, high-risk resilient with no mood disorder or suicidal behavior; HR-SA/MOOD, high-risk with mood disorder and suicidal behavior; HR-MOOD, high-risk with mood disorders and no suicidal behavior.

Table 3. Mixed Effect Model for Weighted 5-HT_{1A} PET Binding, BP_{ND} by Group Controlling for Covariates

	Estimate	Standard Error	P
Amygdala ^b	-0.117	0.014	<.001
Cingulate Cortex ^b	-0.200	0.012	<.001
Dorsolateral Prefrontal Cortex ^b	-0.385	0.013	<.001
Hippocampus ^b	0.441	0.014	<.001
Insula ^b	0.170	0.012	<.001
Medial Prefrontal Cortex ^b	-0.237	0.012	<.001
Occipital Cortex ^b	-0.869	0.015	<.001
Orbitofrontal Cortex ^b	-0.262	0.013	<.001
Parietal Cortex ^b	-0.530	0.013	<.001
Parahippocampal Gyrus ^b	0.222	0.013	<.001
Raphe Nuclei ^b	-0.559	0.014	<.001
Temporal Cortex ^b	0.076	0.012	<.001
Site, Pittsburgh	0.018	0.035	.593
Sex, Males	-0.046	0.036	.198
Age	-0.001	0.002	.760
Race, White	0.065	0.035	.066
HR-R ^a	-0.043	0.046	.348
HR-SA/MOOD ^a	-0.095	0.050	.055
HR-MOOD ^a	-0.095	0.045	.036

Abbreviations: HV, healthy volunteers without a family history of mood disorder or suicidal behavior; HR-R, high-risk resilient with no mood disorder or suicidal behavior; HR-SA/MOOD, high-risk with mood disorder and suicidal behavior; HR-MOOD, high-risk with mood disorders and no suicidal behavior.

^aCompared to healthy volunteers.

^bAnterior Cingulate Cortex used as a reference region.

relationship between PET binding and TSST outcomes. However, we found lower 5-HT_{1A} receptor binding, using BP_p, was associated with higher lethality attempt. We also found higher cortisol reactivity to stress to be associated with higher lethality attempt.

While we found no significant group differences on 5-HT_{1A} receptor BP_{ND} binding in standard or serotonin-specific ROIs, we found small effect sizes in standard BP_{ND} for HR-SA/MOOD (Cohen $d = -0.226$) and HR-MOOD (Cohen $d = -0.226$) compared with HV but not for HR-R. These results suggest that 5-HT_{1A} receptor BP_{ND} binding could be a vulnerability phenotype for the familial transmission of mood disorders and suicidal behavior. Larger studies are needed to detect such a small effect size. However, we found that when suicidal behavior occurs, lower 5-HT_{1A} receptor binding, expressed as BP_p, was significantly associated with higher lethality attempt. A prior study reported no differences in BPND binding among suicide attempters with a range of lethality compared with other high risk and HV groups is consistent with a previous study that used BP_{ND} as an outcome measure (Mann et al., 2019). Another study found an alternative measure of serotonin release and turnover, CSF 5-HIAA, was low only in higher lethality suicide attempters and not in lower lethality suicide attempters, indicating that abnormal serotonin function may only be detectable in those with more lethal suicidal behavior (Mann and Malone, 1997).

However, our results appear to be inconsistent with studies showing higher 5-HT_{1A} receptor binding in those with high-lethality attempts, postmortem studies showing higher binding in those who have died by suicide, and higher binding in RN predicting greater lethality of subsequent suicidal behavior (Sullivan et al., 2015; Oquendo et al., 2016; Underwood et al., 2018). Those studies used a different PET tracer and expressed binding as BP_f. We expressed our results as BP_{ND} because arterial blood sampling was not available for all scans and with that measure could not detect a relationship with the lethality of an attempt. When we used BP_p, we found that higher lethality attempts were associated with lower binding. The difference between BP_p and BP_f is that the latter considers protein binding of the tracer in

the estimation of binding, meaning more protein binding and less free fraction makes the tracer less available to entering the brain and causing an under-estimation of binding. Thus, a difference in protein binding in higher lethality suicide attempters could explain the difference in our results. Suicide attempters in our sample had a lifetime history of attempt and an early age of onset consistent with the familial nature of their phenotype (Brent et al., 2002). Moreover, they had an average lethality of attempt of 2.3, which ranges from 0 to 8, thus corresponding to physical damage requiring medical attention. These results suggest that altered 5-HT_{1A} receptor binding may represent a biomarker for more severe acute suicidal ideation (Oquendo et al., 2016) and higher lethality suicidal behavior, consistent with our earlier results (Sullivan et al., 2015; Oquendo et al., 2016).

Our results also show no significant differences in 5-HT_{1A} binding, when expressed as BP_{ND}, in those with mood disorders with a small effect size (Cohen $d = -0.204$). Prior studies reported no differences in 5-HT_{1A} receptor binding in MDD (Wang et al., 2016) and a study using [11C]WAY-100635 PET scanning, expressed as BP_{ND}, in bipolar disorders (Sargent et al., 2010). However, other studies have reported low 5-HT_{1A} BP_{ND} in MDD (Wang et al., 2016). Patients with recurrent and familial mood disorders were previously found to have low 5-HT_{1A} receptor BP_{ND} compared with healthy controls, in RN and mesiotemporal cortex, and to a lesser extent in other brain regions such as the occipital cortex and postcentral gyrus (Drevets et al., 1999, 2000, 2007; Savitz et al., 2009). In contrast, patients with bipolar disorders were found to have higher 5-HT_{1A} receptor binding using [¹⁸F]FCWAY BP_p, a measure of binding relative to plasma concentrations of PET tracer (Nugent et al., 2013a, 2013b). That finding in bipolar disorder is comparable with our prior work reporting higher 5-HT_{1A} receptor BP_f using [¹¹C]WAY-100635 in patients with current depression or bipolar disorder (Sullivan et al., 2009; Pillai et al., 2018; Zanderigo et al., 2018), healthy high-risk offspring of parents with mood disorders (Milak et al., 2018), as well as in remitted MDD patients (Miller et al., 2009), suggestive of a biological trait that could confer vulnerability to depression. Some of these

discrepancies in terms of the abnormality in depression being high or low binding could be due to discrepancies in PET binding outcome measures (BP_{ND} , BP_p , BP_F , or BP_F/BP_p) and modeling approach for outcome measure estimation, the radioligand used, or the choice of a reference region (Parsey et al., 2010). Other study design factors could also play a role, including small sample sizes, medicated vs unmedicated samples, mood diagnosis, choice of control groups, and other clinical and demographic characteristics. We present our results using BP_{ND} , but we also reported supplementary analyses using BP_p on a subgroup of our sample to allow comparisons with the extant literature.

We found no significant relationship between 5-HT_{1A} receptor binding expressed as BP_{ND} and cortisol responses to stress, but the sample size is modest. We previously reported a negative correlation between baseline salivary cortisol prior to the TSST and 5-HT_{1A} receptor binding, expressed as BP_p , in all brain regions except RN (Steinberg et al., 2019); and 5-HT_{1A} receptor binding, expressed as BP_p , in mesiotemporal cortex was also found to be negatively correlated with plasma cortisol levels (Nugent et al., 2013a). Consistent with these 2 studies, in animal studies, chronic administration of corticosteroids reduced 5-HT_{1A} receptor binding (McAllister-Williams et al., 2001; Man et al., 2002; Fairchild et al., 2003), and attenuation of receptor function was observed in healthy volunteers exposed to repeated cortisol administration (McAllister-Williams et al., 2007).

Although we did not find binding to correlate with cortisol responses to the TSST, we found higher cortisol reactivity to the TSST was associated with higher lethality of SA. These results are consistent with the direction of our results where lower binding was correlated with higher lethality SAs. Lower baseline or resting cortisol levels and total cortisol output throughout the TSST were also associated with higher cortisol reactivity to stress. These results are consistent with prior findings where HPA axis hyperactivity predicts risk for future suicide but is not consistently related to lower lethality suicidal behavior (Mann and Currier, 2007). We also previously reported that baseline and total cortisol output in response to stress were lower in high-risk offspring attempters of parents with mood disorders compared with those without suicide-related behavior and healthy controls (Melhem et al., 2015). We also found this HPA axis profile to be more pronounced among suicide attempters with parental history of attempt, suggesting familial vulnerability, a finding replicated in an independent sample in the United Kingdom (O'Connor et al., 2017). We have extended these findings by showing that this biological profile precedes attempt as determined by assaying hair cortisol concentrations in patients admitted for a suicidal attempt (Melhem et al., 2017). Future studies are needed to better understand how these biological mechanisms implicated in suicidal behavior influence its familial transmission.

There are strengths and limitations of this study. This is the first study, to our knowledge, to examine 5-HT_{1A} receptor binding as a vulnerability and/or resilience phenotype for suicidal behavior and its familial transmission. We studied medication-free individuals to avoid effects of medication on PET results. We also examined standard ROIs and brain regional innervation patterns specific to the serotonin system and found similar results. The most important limitation is our statistical power, which is limited to detect only moderately large effect sizes in terms of Cohen *d* of 0.73 or larger. There were site differences in some characteristics of the study sample, and we observed higher baseline and total cortisol output in participants at the New York site. However, we controlled for site in all analyses. Lastly, while we provided 5-HT_{1A} quantification with 2 different binding outcome measures for comparability with

the literature, we were only able to estimate BP_p in a subset of participants.

In conclusion, this study did not detect a brain region with abnormal 5HT_{1A} receptor binding as a biomarker for familial transmission of mood disorders or suicidal behavior, although it may be related to SA lethality. We have added new evidence linking HPA axis dysfunction to suicidal behavior. Future studies are needed to better understand the biological mechanisms implicated in familial risk for suicidal behavior and how HPA axis function influences such risk.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Interest Statement

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