Ventral prefrontal serotonin 1A receptor binding: a neural marker of vulnerability for mood disorder and suicidal behavior?

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Abstract

Mood disorders and suicidal behavior have moderate heritability and are associated with altered corticolimbic serotonin 1A receptor (5-HT_{1A}) **brain** binding. However, it is unclear whether **this** reflects **genetic effects** or epigenetic effects of childhood **adversity**, compensatory mechanisms, or illness **stress**-related changes. We sought to separate **such** effects on 5-HT_{1A} binding by examining high familial risk individuals (HR) who have passed through the age of greatest risk for psychopathology onset **with and without developing mood disorder or suicidal behavior**. PET imaging quantified 5-HT_{1A} binding potential BP_{ND} using [11C]CUMI-101 in healthy volunteers (HV, N=23) and three groups with one or more relatives **manifesting** early-onset mood disorder and suicide attempt: 1. unaffected HR (N=23); 2. HR with lifetime mood disorder and no suicide attempt (HR-MOOD, N=26); and 3. HR-MOOD with previous suicide attempt (HR-MOOD+SA, N=20). Findings were tested in an independent cohort not selected for family history (HV, MOOD, and MOOD+SA, total N=185). We tested for regional BP_{ND} differences and whether brain-wide patterns distinguished between groups. Low ventral prefrontal 5-HT_{1A} BP_{ND} **was associated with lifetime mood disorder diagnosis and suicide attempt, but only in subjects with a family history of mood disorder and suicide attempt**. Brain-wide 5-HT_{1A} BP_{ND} patterns including low ventral prefrontal and mesiotemporal cortical binding distinguished HR-MOOD+SA from HV. A biological endophenotype associated with resilience was not observed. Low ventral prefrontal 5-HT_{1A} BP_{ND} may reflect familial mood disorder and suicide-related pathology. Further studies are needed to determine if higher ventral prefrontal 5-HT_{1A} BP_{ND} confers resilience, reducing risk of suicidal behavior in the context of **familial risk, and thereby offering** a potential prevention target.

Introduction

Mood disorders including unipolar and bipolar depression (MOOD) and suicidal behavior are associated with morphological brain and serotonin (5-HT) system abnormalities that may mediate genomic liability (1–5). The offspring of individuals with early-onset depression are at higher risk of developing mood disorder and suicidal behavior (6,7). The identification of potentially heritable, biological markers of risk or resilience for mood disorders and suicide is important. Such markers may help to identify persons at elevated risk of developing a mood disorder or attempting suicide and thereby, can facilitate prevention efforts.

The pathology of mood disorders and suicide risk have been consistently linked to alterations in brain serotonin 1A receptor (5-HT_{1A}) binding potential using Positron Emission Tomography (PET) and postmortem brain studies of suicide decedents. Most studies demonstrating lower corticolimbic 5-HT_{1A} receptor binding in depressed patients have expressed the binding in terms of binding potential BP_{ND} (5,8). We have previously reported that binding potential BP_F was elevated in depressed patients both during an acute episode (9,10) and while in remission, suggesting this is a biologic trait (11). Subsequently, we reported a pilot study that suggested altered brain 5-HT_{1A} binding potential may be familially transmitted from depressed parents to their children and may be an endophenotype differentiating individuals at higher risk for developing major depressive disorder (12). To our knowledge, this was the first study to examine brain 5-HT_{1A} binding potential in a small sample of unaffected 1st degree relatives of individuals with a mood disorder and lifetime history of suicide attempt. This study suggests that 5-HT_{1A} receptor binding abnormalities are not purely a consequence of illness or treatment. However, **a paucity of** PET studies of the serotonin system in unaffected relatives **means it is unknown** whether alterations in brain 5-HT_{1A} binding potential are familial traits that confer risk or resilience for developing a mood disorder and suicidality.

Notably, the current study used a different radioligand than our previous related work mentioned above. Regarding differences in radiotracers, [^{11}C]WAY-100635, used in our previous papers (9–12), is a 5-HT_{1A} antagonist that bind **with equal affinity to the coupled and uncoupled conformations**, while [^{11}C]CUMI-101, used here, **as a biased agonist**, may predominantly bind only to receptors in the high affinity **conformation** (13,14). **An agonist and an antagonist tracer** may measure different 1A receptor spatial distributions and binding affinity.

To distinguish differences associated with risk for developing psychiatric disorder from differences related to compensatory adaptation possibly marking resilience or illness related changes, the current study examined regional differences in regional 5-HT_{1A} binding potential using [^{11}C]CUMI-101 in low risk healthy volunteers (HV) and 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. High risk individuals were either unaffected **despite being HR and** recruited when largely beyond the age of risk for mood and suicidal behavior), or affected with lifetime history of mood disorder alone (HR-MOOD) or mood disorder plus one or more lifetime suicide attempts (HR-MOOD+SA). Based on previous findings from our group and others (5,9–11,15,16), we hypothesized altered **regional brain** 5-HT_{1A} receptor binding potential in both HR-MOOD and HR-MOOD+SA relative to HV, and **explored whether** HR would display different alterations suggesting a biological phenotype related to resilience for mood disorders or suicidal behavior. We also tested whether statistical learning applied to whole-brain patterns of 5-HT_{1A} receptor binding potential could distinguish HVs from unaffected and affected high risk individuals. This complementary analysis increased power to detect and characterize patterns of brain-wide abnormalities in 5-HT_{1A} receptor binding potential that may

further elucidate the pathophysiology of mood disorder and suicidal behavior. Positive findings may have clinical utility in identifying high risk individuals before onset of psychopathology.

To address these objectives, an exploratory, voxel-wise analysis was conducted in the primary “discovery” dataset (FAMPATH) to **identify candidate clusters showing group differences in 5-HT_{1A} receptor binding potential**. These clusters were then **employed as regions of interest (ROIs) to be tested for replication in ROI-level analyses** in an independent dataset of healthy volunteers and individuals affected with current or lifetime mood disorder and often also lifetime history of suicide attempt, that was available from two additional studies using the same [11C]-CUMI-101 PET tracer. The subjects in this dataset had current or lifetime history of major depressive disorder and were not recruited with the stringent family history requirements for FAMPATH. Similarly, multivoxel pattern analysis (MVPA) was applied to voxel-wise PET estimated parametric maps of binding potential to distinguish between the four groups in the primary discovery FAMPATH dataset (HV, HR, HR-MOOD, and HR-MOOD+SA), and the best performing model was applied to the second independent dataset to test its generalizability. Finally, we compared the results to those in the HR group to infer whether observed differences reflected putative markers of risk, resilience or compensatory mechanisms for developing a mood disorder and suicidal behavior.

Methods and Materials

Inclusion/exclusion criteria for the primary dataset: FAMPATH

The FAMPATH sample consisted of high-risk individuals and healthy volunteers (HV) who were 25 years of age or older, an age by which mood disorder and suicidal behavior in our familial transmission studies are generally phenotypically clinically expressed (17,18). All high-risk subjects were categorized as being at-risk for a mood disorder and suicide because

they had a first or second degree relative with early-onset (<40 years) mood disorder and a lifetime history of suicide attempt. The subjects in the high-risk group were separated into vulnerable and resilient groups based on their phenotype: individuals with a lifetime history (current or past) of mood disorders and a history of suicide attempt (HR-MOOD+SA, n=20); individuals with a lifetime history (current or past) of mood disorders but no suicide attempt (HR-MOOD, n=26); and resilient (HR, n=23) individuals with no personal history of mood disorder or suicide attempt. Qualifying mood disorders included major depressive disorder, bipolar disorder, dysthymia and depressive disorder not otherwise specified. The HR-MOOD sample consisted of 22 participants with major depressive disorder and 4 participants with other diagnoses. The HR-MOOD+SA sample consisted of 16 participants with major depressive disorder, and 4 with bipolar disorder. Healthy volunteers (HVs, n=23) had no personal or family history of psychiatric disorders or suicidal behavior. The sample was recruited at two sites, University of Pittsburgh Medical Center and New York State Psychiatric Institute, in protocols approved by the respective Institutional Review Boards (IRB) at the University of Pittsburgh and The New York State Psychiatric Institute. Participants were recruited through outpatient clinics, clinician referrals and IRB-approved advertisements and all participants provided written, informed consent. See Supplementary Methods for assessments and additional inclusion/exclusion criteria for FAMPATH and the second independent dataset.

Final sample sizes across the three included studies

The primary discovery dataset (FAMPATH) consisted of 23 healthy volunteers (HV), 23 high familial risk (HR) participants, 26 HR with a lifetime history of mood disorder (HR-MOOD), and 20 HR-MOOD with one or more lifetime suicide attempts (HR-MOOD+SA). The second independent dataset included 36 HV, 38 individuals with current mood disorder (MOOD), and 19 participants with MOOD and one or more lifetime suicide attempts (MOOD+SA). The total

sample size of the second independent dataset is $N=93$, while the total across both sets is $N=185$. Note that data in the second dataset was acquired and analyzed the same way as was the FAMPATH cohort in terms of PET imaging acquisition, binding potential quantification and subsequent analysis. Our group has not previously published on 5-HT_{1A} PET imaging findings in this second cohort.

PET/MR scanning, image preprocessing and binding quantification

See Supplementary Methods for PET/MR scanning and imaging preprocessing procedures. PET procedures to quantify 5-HT_{1A} binding potential BP_{ND} were described in detail elsewhere (19). Our primary outcome measure is 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 are unoccupied or available to bind to the tracer, and K_D is the tracer equilibrium dissociation constant. BP_{ND} is used as its quantification does not require blood sampling. BP_{ND} was quantified at the voxel level using the reference tissue version of the likelihood estimation in graphical analysis (19) 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 (19). See Supplementary Methods for quantification of BP_P and V_T for subjects with usable venous samples (~65% of the total sample).

Imaging processing and analysis

Voxel-wise univariate analyses: Analyses were conducted using SPM12 (www.fil.ion.ucl.ac.uk/spm/) and implemented in MATLAB version 7.13 on Ubuntu Linux OS 14.04. Voxel-level PET binding maps were spatially normalized to a 2 x 2 x 2 mm MNI-space template using Advanced Normalization Tools (ANTs) (20) and smoothed with a 8 mm full width at half maximum FWHM Gaussian filter. The images were submitted to a 1-way

ANCOVA with four levels (HV, HR, HR-MOOD, HR-MOOD+SA) in the primary FAMPATH dataset and three levels (HV, MOOD, MOOD+SA) in the second dataset. An absolute threshold was applied to remove voxels with BP_{ND} values below 0.05, and non-gray matter voxels were excluded from analyses via a gray matter mask generated by thresholding a tissue probability map in MNI space (provided with SPM8) at > 0.2 . Sex, age and scan site were included as nuisance covariates for both datasets.

A cluster-determining threshold (CDT) of $p < 0.01$ and $k > 150$ was used to identify candidate regions in the FAMPATH primary discovery dataset that differed between groups (HV, HR, HR-MOOD, and HR-MOOD+SA). These clusters were then used to define ROIs for ROI analysis in the second independent dataset. Two types of ROI analyses were conducted for each of the clusters identified in the primary discovery dataset. The first analysis used Marsbar v0.44 (<http://marsbar.sourceforge.net>) and the signal (i.e. voxel-wise beta estimates from 1st level analyses) within each ROI was first averaged together before model estimation. **In the second analysis, an anatomical ROI mask generated using WFU Pickatlas, was used for small volume correction (SVC) in which models are estimated across all voxels within the mask. For the latter, correction for multiple comparisons was conducted using 3dClustSim (compiled December 11, 2018) with the -acf option (input parameters estimated using residuals from the SPM design file) and a cluster determining threshold of $p < 0.05$ for cluster sizes defined using $N=1$ nearest neighbors and 2-sided testing. This approach was based on recent recommendations to reduce false-positive rates when using cluster-extent correction (21,22) and includes a more accurate estimate of the noise smoothness values using a mixed model (Gaussian plus a monoexponential) (23). Results were also confirmed using nonparametric permutation for cluster-extent**

correction (FSL's *randomise* function with 2000 permutations and cluster determining threshold $t\text{-value}=1.96$).

Voxel-wise multivariate statistical learning analyses: Multivoxel pattern analysis (MVPA) analyzed the joint BP_{ND} signal across multiple regions in a single subject in order to predict the diagnostic class of that subject. Briefly, binary classification tasks using a linear kernel Support Vector Machine SVM (24) with a filter feature selection (t -test) and leave-one-out cross-validation was applied using the Spider v1.71 MATLAB toolbox (<http://people.kyb.tuebingen.mpg.de/spider/>) with default regularization parameter $C = 1$. Classification performance for each top N selected features (voxels) was assessed using non-parametric permutation. See Supplementary Methods for more details.

The above procedures were repeated within the FAMPATH and second independent dataset for each pairwise group comparison within each cohort. In addition, to test whether the models learned in the FAMPATH dataset are generalizable to individuals not selected for family history, a model trained over the full FAMPATH sample (using the top N features that gave the best classification performance) was applied to the second independent dataset.

Results

Demographic and clinical results

Within both the FAMPATH and second dataset, the subgroups did not differ in sex ratio ($p\text{-values} > 0.2$). In the second dataset the MOOD+SA were younger, and the MOOD older, **compared with** the HV group (ANOVA omnibus $p < 0.001$, see Table 1). The second dataset had a smaller MOOD+SA group because one study from which the sample was drawn did not actively recruit individuals with one or more lifetime suicide attempts. The HR-MOOD and HR-MOOD+SA groups in the primary FAMPATH dataset had relatively lower current Beck Depression Inventory (BDI) and Hamilton Depression Rating Scale (HAM17) scores than in the

second dataset. Participants in the FAMPATH were not required to be currently ill because we previously reported (11) that altered 5-HT_{1A} binding was a trait that was present during acute depressive episodes and when in remission between episodes (Table 1). The second dataset MOOD and MOOD+SA subgroups had higher BDI and HAM17 scores because the sample involved currently depressed subjects (Table 1).

As expected, the FAMPATH HR-MOOD and HR-MOOD+SA subgroups had higher rates of familial mood disorder relative to the second dataset which was not selected for family history (85-94% vs. 47-53% subjects with one or more first degree relatives with major depressive disorder). They also had higher rates of familial suicidal behavior (~71-84% vs. 6-16% of subjects with one or more first degree relatives with lifetime suicide attempt). Among the HR subgroup of FAMPATH, 82% had at least one first degree relative with MDD, and 82% had at least one first degree relative who made a nonfatal suicide attempt; the remainder had at least one second degree relative with lifetime MOOD and suicide attempt (see Table 1).

Voxel-based 5-HT_{1A} Receptor Binding Univariate Analyses

A **whole-brain** omnibus F-test thresholded at $p < 0.01$, $k > 150$ testing for differences between the four groups (HV, HR, HR-MOOD and HR-MOOD+SA) in the FAMPATH dataset **revealed** two clusters: a cluster in the ventromedial prefrontal cortex (vmPFC, Figure 1A, top row) and a rostral cluster in medial orbitofrontal cortex (mOFC, see Figure 1A, bottom row). Post hoc t-tests show lower 5HT_{1A} BP_{ND} in vmPFC and mOFC in HR-MOOD vs. HV ($t(85)=3.12$, $p=0.001$ and $t(85)=3.67$, $p=0.0002$), HR-MOOD+SA vs. HV ($t(85)=3.50$, $p=0.0004$ and $t(85)=3.08$, $p=0.001$), HR-MOOD vs. HR ($t(85)=2.44$, $p=0.008$ and $t(85)=2.27$, $p=0.01$), and HR-MOOD+SA vs. HR ($t(85)=2.83$, $p=0.003$ and $t(85)=1.75$, $p=0.04$) (see Table 2).

The two clusters were used as ROIs to test for group differences (HV, MOOD, MOOD+SA) in the second dataset. In this dataset, an omnibus F-test found no evidence for

group differences in either cluster (p-values > 0.2, Figure 1A, middle panel). Since the participants in this cohort were not selected for family history (in contrast to FAMPATH) we tested whether group differences might emerge when the participants were stratified by familial risk (“HR”) status. For this analysis in the second dataset, **5 subjects (2 MOOD and 3 MOOD+SA) had at least one first-degree relative with MDD and at least one 1st degree relative with lifetime suicide attempt (HR-patients, see Table 1).** The median 5-HT_{1A} BP_{ND} in the vmPFC and mOFC clusters for the 5 subjects was compared to all subjects in the second dataset without such family history (HV and non-HR MOOD and MOOD+SA, or non-HR, patients). No significant differences in 5-HT_{1A} BP_{ND} were found in either cluster (vmPFC: HR-patients median=0.77, HV and non-HR patients median=0.88, Mann-Whitney U test p=0.16; mOFC: HR-patients median=1.00, HV and non-HR patients median=1.08, Mann-Whitney U test p=0.37, see Figure 1A, right panel). We also tested for lower binding in positive family history subjects in ventral prefrontal cortex using voxel-wise testing constricted to an anatomical ROI mask and small volume cluster-extent correction. This analysis revealed a cluster in ventral prefrontal cortex in the vicinity of subgenual anterior cingulate cortex that showed lower 5-HT_{1A} BP_{ND} in positive family history subjects relative to HV and non-HR patients that was statistically significant in a one-tailed comparison (p=0.07 cluster extent corrected, Figure 1B, right).

Voxel-based multivariate learning analysis results

Multivoxel pattern analysis (MVPA) was applied to distinguish between the four diagnostic groups in the primary FAMPATH dataset using the whole-brain, voxel-wise 5-HT_{1A} binding potential PET maps. Of the six total pairwise comparisons (HV vs. HR, HV vs. HR-MOOD, HV vs. HR-MOOD+SA, HR-MOOD vs. HR-MOOD+SA, HR vs. HR-MOOD and HR vs. HR-MOOD+SA), significant classification performance was achieved for HR-MOOD+SA vs.

HV ($p < 0.001$ corrected). The peak performance was attained when using the top 200 features/voxels (AUC=0.74, sensitivity=0.78, specificity=0.70, Figure 2, left panel). Regions that figured heavily into the discrimination included medial orbitofrontal cortex, ventromedial prefrontal cortex, inferior and anterior temporal cortex (Figure 2, right panel). A best performing model was trained over the full FAMPATH dataset using the top 200 selected features (voxels). The learned model was then applied to the second dataset to classify HV vs. MOOD+SA. Classification performance was greater than the theoretical null classification (AUC=0.55) but did not reach statistical significance ($p=0.15$, data not shown).

Discussion

Across two independent datasets, we observed evidence for lower 5-HT_{1A} BP_{ND} receptor binding in high familial risk MOOD and MOOD+SA (vs. HV) individuals in ventromedial prefrontal and medial orbitofrontal cortex. The directionality of these findings is consistent with studies by other groups that have reported lower 5-HT_{1A} binding potential BP_{ND} in limbic, frontal and temporal cortices in major depression (15,25–27) and in remitted depressed patients (28). Our findings are also consistent with a 2016 meta-analysis of 10 studies (including 218 patients with MDD and 261 HV) that found lower 5-HT_{1A} receptor binding potential BP_{ND} in various corticolimbic ROIs, although vmPFC and mOFC did not appear to be included in the meta-analysis (5). In the current study, low ventral prefrontal 5-HT_{1A} BP_{ND} binding potential was found in groups with mood disorder alone or with mood disorder and suicidal behavior, but only in individuals with a family history of both mood disorder and suicidal behavior. Therefore, low ventral prefrontal 5-HT_{1A} BP_{ND} may reflect suicide- and mood disorder-related neuropathology in the context of high familial risk for suicide.

A related paper by our group (Melhem et al. 2021) that used the same FAMPATH cohort found no group differences in $5\text{-HT}_{1A} \text{ BP}_{ND}$ (29). The key methodological difference with Melhem et al. 2021 is that this study estimated and examined group differences in *voxel-wise* $5\text{-HT}_{1A} \text{ BP}_{ND}$, whereas Melhem et al. 2021 used *ROI-level quantification of 5-HT_{1A} BP_{ND}* in a priori regions, where only the average binding within a specified region of interest is estimated. **Melhem 2021 et al. did not detect group differences in any of the ROIs tested.** An anatomical ROI-based analysis is less likely to detect group differences if the signal is confined to a relatively small area within a given ROI parcellation, since the signal would be diluted by other voxels within the ROI that do not show a group difference. This problem is worse for larger ROIs like the prefrontal cortex.

The vmPFC and mOFC are involved in depression-relevant processes, including emotional regulation, reward processing, and negative self-appraisal (30) and have been previously implicated with pathophysiology of both mood disorders and suicide across a range of analysis techniques including neuroimaging, neuropathologic and lesion analysis (31,32). Our findings also converge with a recent comprehensive review of two decades of suicide neuroimaging studies, that suggests that impairments in ventral prefrontal cortex play a role in suicide ideation and development of suicidal behavior (4).

According to a model proposed in (33), neural differences shared between affected and unaffected HR individuals compared to low-risk HV are putative markers of risk, whereas neural changes differentiating affected and unaffected HR may reflect compensatory, protective, or resilience markers. In our primary FAMPATH dataset, the $5\text{-HT}_{1A} \text{ BP}_{ND}$ in vmPFC and mOFC was lower in HR-MOOD and HR-MOOD+SA compared with HR, and the HR levels were comparable to those observed in the low-risk HV group (see Table 2). Moreover, the MVPA did not distinguish HR from the HV group. Therefore, low ventral prefrontal $5\text{-HT}_{1A} \text{ BP}_{ND}$

is not an abnormality associated with familial risk or resilience since it is absent in the HR group.

Low ventral prefrontal 5-HT_{1A} BP_{ND} may have a causal relationship or be part of the pathogenesis of MOOD and MOOD+SA **in individuals with familial risk**. Because we **did not** observe lower ventral prefrontal 5-HT_{1A} BP_{ND} in MOOD+SA without a family history, it appears that **low 5-HT_{1A} BP_{ND} in this brain region may mediate familial causal factors responsible for MOOD and MOOD+SA**. It should be noted that without a longitudinal study of high-risk individuals that shows these biologic changes are present before the development of symptoms, one cannot rule out the possibility that these brain changes in binding are a consequence of the illness in terms of being either damage due to the illness or a homeostatic response. However, **that would not explain the familial transmission and we** previously reported altered 5-HT_{1A} binding in the brains of asymptomatic offspring of depressed parents and those with the most abnormal binding went on to develop a subsequent depression (12). If the findings of this pilot study can be replicated and generalized to MOOD+SA, that could rule out homeostatic responses or biologic change as a response to active illness as an explanation of our findings. Further studies are needed to determine if higher 5-HT_{1A} binding potential in vmPFC/mOFC or other brain regions may confer resilience to developing suicidal behavior in the context of mood disorder. If so, it could be a potential mood disorder-suicide prevention target.

Consistent with the univariate analysis which found the largest and most consistent group differences between HV and HR-MOOD+SA, MVPA distinguished between HV vs. HR-MOOD+SA with above chance performance, with informative voxels located in inferior mesiotemporal cortex and vmPFC/mOFC. The MVPA could not distinguish between any of the other group pairs. Moreover, the best fitting model for HV vs. HR-MOOD+SA did not

distinguish HV vs. MOOD+SA in the replication dataset. A possible reason that MVPA could not distinguish between the other group pairs and that the FAMPATH HV vs. HR-MOOD+SA model did not generalize to the CONTE HV vs. MOOD+SA dataset is that the MVPA picked up signals in the brain-wide 5-HT_{1A} binding potential patterns that were specific to having high familial risk for both mood disorder and suicidal behavior. Future studies of HR-MOOD+SA are required to test whether our multivariate pattern classification results generalize to other samples.

Limitations

Previous studies, including by our group, have reported findings that contradict the findings of the current study. Specifically, we previously reported higher 5-HT_{1A} receptor binding potential BP_P and/or BP_F during a major depressive episode (9,10), in remitted depressed patients when between episodes and unmedicated (11), and in HR offspring of depressed patients (Milak et al., 2018). The discrepancy with our current results is likely explained by differences in outcome measure (8,34) and choice of reference region (white matter cerebellum for our previous results vs. gray matter cerebellum here), and by differences in the characteristics of the radiotracers used to quantify 5-HT_{1A} binding potential. Our previous studies used BP_F, while this study used BP_{ND} as the primary outcome measure since only venous plasma measures, required for calculation of BP_F, BP_P and V_T outcomes, were available, and were available only for a subset (~65%) of the subjects and we wanted to maximize sample sizes. When using the subset of subjects in FAMPATH with BP_P and V_T images, the main findings (higher 5-HT_{1A} binding in HV and HR vs. HR-MOOD and HR-MOOD+SA) were consistent with results obtained using BP_{ND} in the full sample in terms of the direction of the findings, but with higher p-values (HV+HR > HR-MOOD+HR-MOOD+SA, p-values between 0.04 and 0.25 for BP_P and V_T, data not shown).

Our MVPA analysis demonstrates alterations in the pattern of brain-wide 5-HT_{1A} BP_{ND} that distinguishes HR-MOOD+SA from low risk HV. The model did not generalize to MOOD+SA with low familial risk in the second dataset. Future studies with larger sample sizes are needed to determine if our findings can be extended to lower risk suicidal behavior in the context of mood disorder.

Finally, we did not find direct evidence for a risk or resilience biological endophenotype. It is possible that other biologic systems, perhaps not involving the serotonin system, provide the resilience seen clinically in the HR group we identified who had no evidence of having developed mood disorder or manifested suicidal behavior.

Conclusion

In summary, our findings suggest **lower ventral prefrontal and orbitofrontal 5-HT_{1A} BP_{ND} in individuals with both lifetime and family history of suicide attempt and mood disorder. Differences in ventral prefrontal 5-HT_{1A} BP_{ND} were not observed in patients without family history for these conditions, suggesting that both high familial risk and psychopathology account for the finding.** Whole-brain pattern classification analysis showed promise in distinguishing healthy volunteers from both high familial risk suicide attempters with mood disorder, suggesting suicidal behavior in the context of mood disorder and familial risk is associated with altered spatial pattern of 5-HT_{1A} BP_{ND} in ventromedial prefrontal and mesiotemporal cortex. If our results are confirmed in future studies, low ventral prefrontal 5-HT_{1A} BP_{ND} may be a biomarker of risk for suicidal behavior and mood disorders. **Future studies could then seek the biological phenotype of resilience and consider if modifying binding in this brain region can reduce the risk of familial transmission of depression and suicidal behavior.** 5-HT_{1A} BP_{ND} could be targeted with pharmacological and/or cognitive behavioral interventions. For example, Gray et al. (35) found chronic SSRI

treatment alters 5-HT_{1A} receptor binding potential. Future longitudinal studies should also examine temporal relationships between suicidal behavior, mood disorder course, and ventral prefrontal serotonin 1A binding.

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Conflicts of interest

Authors SPP NMM FZ ML EB JMM have no conflicts of interest to report. JJM and AB receive royalties for commercial use of the C-SSRS from the Research Foundation for Mental Hygiene. DAB receives royalties from the electronic self-rated version of the C-SSRS from eRT, Inc.

Data availability statement

The derived data that support the findings of this study are available upon reasonable request from the corresponding authors. The raw data are not publicly available as participants of this study did not agree for their data to be shared publicly.

Table 1. Demographics and behavioral data. Group differences were assessed using Kruskal–Wallis one-way ANOVA (continuous variables) or chi² test (categorical variables).

Note that FAMPATH required HR participants have a first-degree or second-degree relative with *both* a qualifying mood disorder and history of suicidal behavior. The FamDep and FamSui variables indicate number of participants with one *or* more first-degree relatives with MDD or suicide attempt, respectively. **FamDep and FamSui are reported here since these variables were the same and accessible across both datasets.**

iqr=interquartile range.

FAMPATH	HV (N=23)	HR (N=23)	HR-MOOD (N=26)	HR-MOOD+SA (N=20)	pval
Age: mean (iqr)	39.91 (15.25)	39.22 (20.50)	36.46 (15.00)	37.30 (17.00)	0.62
HAM17: mean (iqr)	0.91 (1.75)	1.13 (1.75)	4.04 (6.00)	10.30 (12.50)	0
BDI: mean (iqr)	1.35 (1.75)	2.35 (4.75)	6.69 (10.00)	17.35 (15.00)	0
Sex: count (%)	7/16 (0.30/0.70)	7/16 (0.30/0.70)	9/17 (0.35/0.65)	7/13 (0.35/0.65)	0.98
FamDep: count (%)	23/0 (1.00/0.00)	4/18 (0.18/0.82)	4/22 (0.15/0.85)	1/16 (0.06/0.94)	0
FamSui: count (%)	23/0 (1.00/0.00)	4/18 (0.18/0.82)	4/21 (0.16/0.84)	5/12 (0.29/0.71)	0
Site: count (%)	13/10 (0.57/0.43)	7/16 (0.30/0.70)	10/16 (0.38/0.62)	9/11 (0.45/0.55)	0.33
SECOND DATASET	HV (N=36)	N/A	MOOD (N=38)	MOOD+SA (N=19)	pval
Age: mean (iqr)	32.14 (13.00)		35.74 (18.00)	27.21 (12.50)	0
HAM17: mean (iqr)	0.92 (1.00)		17.63 (6.00)	17.00 (11.00)	0
BDI: mean (iqr)	0.92 (1.00)		25.11 (9.00)	23.22 (6.00)	0
Sex: count (%)	14/22 (0.39/0.61)		16/22 (0.42/0.58)	8/11 (0.42/0.58)	0.95
FamDep: count (%)	35/0 (1.00/0.00)		16/18 (0.47/0.53)	10/9 (0.53/0.47)	0
FamSui: count (%)	31/0 (1.00/0.00)		31/2 (0.94/0.06)	16/3 (0.84/0.16)	0.07
site: count (%)	7/29 (0.19/0.81)		10/28 (0.26/0.74)	1/18 (0.05/0.95)	0.17

Table 2. Post hoc t-tests for pairwise contrasts between groups using ROI analysis (Marsbar, see methods). vmPFC=dorsal cluster in Figure 1, top row; mOFC=ventral cluster in Figure 1, bottom row; **All reported p-values are one-sided given that we hypothesized specific directions in the second independent dataset based on FAMPATH dataset results.**

STUDY AND ROI	Contrast 1		Contrast 2		Contrast 3		Contrast 4		Contrast 5		Contrast 6	
<u>FAMPATH</u>	HV > HR		HV > HR-MOOD		HV > HR-MOOD+SA		HR > HR-MOOD		HR > HR-MOOD+SA		HR-MOOD > HR-MOOD+SA	
	<i>t(85)</i>	<i>p-val</i>	<i>t(85)</i>	<i>p-val</i>	<i>t(85)</i>	<i>p-val</i>	<i>t(85)</i>	<i>p-val</i>	<i>t(85)</i>	<i>p-val</i>	<i>t(85)</i>	<i>p-val</i>
vmPFC	0.69	0.25	3.12	0.001	3.50	0.0004	2.44	0.008	2.83	0.003	0.57	0.29
mOFC	1.37	0.09	3.67	0.0002	3.08	0.001	2.27	0.01	1.75	0.04	-0.40	0.65
<u>SECOND DATASET</u>	HV and non-HR patients > "HR"*		HV > MOOD		HV > MOOD+SA		N/A		N/A		MOOD > MOOD+SA	
	<i>diff</i>	<i>p-val</i>	<i>t(87)</i>	<i>p-val</i>	<i>t(87)</i>	<i>p-val</i>					<i>t(87)</i>	<i>p-val</i>
vmPFC	0.11	0.16	0.21	0.42	-0.14	0.56					0.11	0.46
mOFC	0.08	0.37	-0.71	0.76	0.29	0.39					0.42	0.34

*See Figure 1A (right panel) and Figure 1B for voxel-wise results when using an anatomic ROI and small volume cluster-extent correction.

Figure 1

B**2nd dataset (by HR status)**HV and non-HR patients > HR patients ($p < 0.05$ uncorrected, $k > 0$)

Region	Coordinates (MNI)			k	t
	X	Y	Z		
Subgenual anterior cingulate cortex	6	14	-22	283*	2.85
vmPFC	0	54	-16	173**	2.25
	10	38	-8	66	2.12

* $p = 0.07$ and ** $p = 0.18$ cluster-extent corrected

Figure 2.

Figure Legends

Figure 1. Individuals with both lifetime and family history of mood disorder and suicide attempt have lower 5-HT_{1A} binding in ventromedial prefrontal and orbitofrontal cortex with respect to healthy volunteers and patients without family history for these traits. A)

A voxel-wise, whole brain omnibus F-test in the discovery dataset (FAMPATH) identified two clusters with different 5-HT_{1A} binding potential (BP_{ND}) among four groups: 1. healthy volunteers (HV, N=23) and three groups with one or more relatives with early-onset mood disorder and suicide attempt: 2. unaffected high risk individuals (HR, N=23), 3. individuals with lifetime mood disorder (HR-MOOD, N=26), and 4. HR-MOOD individuals with lifetime history of suicide attempt (HR-MOOD+SA, N=20) ($p < 0.01$ uncorrected, $k > 150$, **left column**). One cluster was in ventromedial prefrontal cortex (vmPFC, **top row**), and the other was located more ventrally in medial orbitofrontal cortex (mOFC, **bottom row**). Each cluster was used to define ROIs for ROI-level analyses in the second independent dataset (**middle column**). **The second dataset included HV (N=36), individuals with mood disorder (MOOD, N=38), and depressed individuals with one or more lifetime suicide attempt (MOOD+SA, N=19). Group differences in 5-HT_{1A} binding potential in either cluster were not observed in this dataset (p -values > 0.2 uncorrected). Five of the MOOD and MOOD+SA subjects in this second dataset were considered “high familial risk” (“HR-patients”) on the basis of having at least one first degree relative with MDD and at least one first degree relative with lifetime suicide attempt. The HV and HR non-patients combined had higher median 5-HT_{1A} binding potential than the 5 HR-patients in both regions, though neither cluster reached statistical significance based on Mann Whitney U-test (right column). B)** An anatomical mask defined using the WFU Pickatlas (left) was used for a voxel-wise small volume cluster-extent corrected ROI analysis. Lower 5-HT_{1A} binding potential in the

replication sample positive family history-subjects vs. HV and non-familial depressed and suicide attempter subjects was observed in subgenual anterior cingulate cortex at a one tailed level of statistical significance ($p=0.07$ corrected, right).

Figure 2. Voxel-wise PET binding distinguishes healthy volunteers from depressed suicide attempters. (A) Out-of-sample classification performance (AUC) in the primary FAMPATH dataset was plotted vs. number of features that have been ranked by their absolute *t*-score (in the training data). Three of the six total pairwise comparisons are plotted (HV vs. HR-MOOD, HV vs. MOOD+SA, and HR-MOOD vs. HR-MOOD+SA, the rest are not shown). Above chance classification for HV vs. MOOD+SA was achieved across all top N features ($p<0.001$ corrected for multiple comparisons), with peak performance when using top 200 selected features (highlighted box, AUC=0.74, sensitivity=0.78, specificity=0.70). Solid gray line plots the mean of the null distribution and error bars represent the spread such that 95% of the null values fall within the lower and upper bounds). **(B)** The top 200 features (voxels) over the entire training (primary FAMPATH) dataset were used to train a classification model and plot the informative features on the brain. Informative regions included medial orbitofrontal cortex, ventromedial prefrontal cortex, inferior and anterior temporal cortex (top two rows). Each voxel was color coded according to its weight score from the SVM model learned from the full dataset using the top 200 features. Red indicates positive weights, blue indicates negative weights.

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Supplemental Material for “Ventral prefrontal serotonin 1A receptor binding: a neural marker of vulnerability for mood disorder and suicidal behavior?”

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Contents:

Supplementary Methods

Supplementary References

Running title: Serotonin 1A receptor binding and familial suicide risk

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Supplementary Methods

Inclusion/exclusion criteria and assessments

We required all subjects to be off psychotropic medication and other serotonergic medications for at least 3 weeks, and off any anticoagulant medication for a minimum of 10 days. The NYC site allowed individuals to enroll if they were taking medication that was ineffective after a trial of adequate dose and duration. Those individuals underwent medication taper followed by 3-week medication washout prior to scanning. We permitted use of benzodiazepines up to 3 days prior to scanning. Exclusion criteria consisted of: other Axis I psychiatric diagnosis, current alcohol or drug use disorder, history of IV drug use or ecstasy use more than twice, family history of schizophrenia, significant active physical illness, lacking capacity to consent, pregnancy, presence of metal implants or a medicinal patch, medical or occupational radiation exposure within the past 12 months, or a head injury causing loss of consciousness for more than three minutes or measureable memory impairment.

Psychiatric diagnoses were assessed in all groups including HVs using the Structured Clinical Interview for DSM-5 disorders (SCID-5) (1) by an experienced clinician with at least a Master's degree and on the basis of other data such as past medical records, and were reviewed by senior clinicians at a consensus conference. Familial history of psychiatric disorders was assessed using the FH-RDC (2). Suicidal behavior was assessed using the Columbia Suicide History Form and the Columbia Suicide Severity Rating Scale (C-SSRS) (3) and depressive symptoms were assessed using the Hamilton Depression Rating Scale (HAMD) (4). Self-reported symptoms were assessed using the Beck Depression Inventory (BDI) (5) and the Beck Hopelessness

Inventory (BHI) (6). We also assessed impulsivity and aggression using the Brown-Goodwin Lifetime History of Aggression Scale (7) and the Barratt Impulsivity Scale (BIS-II) (8), respectively. Childhood abuse and neglect was assessed using the Childhood Trauma Questionnaire (CTQ) (9).

Inclusion/exclusion criteria: Second independent dataset

The second dataset was comprised of the two separate PET studies run on the same scanner. The first study (“CONTE”) enrolled individuals with current major depressive disorder (MDD), with and without a history of past suicide attempt (MOOD and MOOD+SA), as well as healthy volunteers (HV). MOOD and MOOD+SA groups had a 17-item Hamilton Depression Rating Scale of ≥ 16 at the time of enrollment. Other eligibility criteria and assessments were consistent with the FAMPATH sample except for the family history requirements and psychiatric illness assessment used SCID-1 for DSM-4 disorders (10).

The second study (Neurobiology of Depression and Antidepressant Action, or NODA) participants were individuals with current MDD with a current 17-item HDRS score ≥ 16 ; remitted MDD with 17-item HDRS score < 10 , in remission for ≥ 1 year and off antidepressants and mood stabilizers for ≥ 6 months; and HVs. For the current analysis, current and remitted MDD participants were combined, and categorized by suicide attempt history into MOOD and MOOD+SA groups. Other eligibility criteria and assessments were consistent with the FAMPATH sample except for the family history requirements and psychiatric illness assessment used SCID-1 for DSM-4 disorders (10).

PET/MR scanning and image preprocessing

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 scanner in NYC and Pittsburgh (Siemens/CTI, Knoxville, TN, USA). [11C]CUMI-101, synthesized as described by our laboratory (11–13), was injected as an intravenous bolus over 30 seconds. Emission data were collected in 3-D mode for 120 minutes using 21 frames of increasing duration (3 x 20 sec, 3 x 1 min, 3 x 2 min, 2 x 5 min, 10 x 10 min). The NODA dataset includes data from two sites: the first used the same NYC scanner mentioned above and a second site used an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, Tennessee) at Cornell University.

MRI image acquisition: The Pittsburgh and NY sites have standardized MRI protocols. MRI images were acquired on a GE 3 Tesla MR750 scanner utilizing 18 high order superconducting shim coils and 5 resistive shim coils, and acquiring images using a 32-channel head-coil 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 (TR), 6 ms; echo time (TE), 2.4 ms; flip angle, 8°; 1-mm slice thickness (zero gap); 178 slices; and field of view, 256 x 256mm. All images were reconstructed to a size of 256 x 256, with an isotropic resolution of 1mm³. This T1-weighted structural image was used for identification of regions of interest, gray-white matter segmentation, skull-stripping, and for PET co-registration.

Image preprocessing: Image preprocessing was performed in MATLAB (The Mathworks, Natick, MA, USA). Head motion was corrected by registering each PET frame to frame eight 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 of the PET images, using FLIRT with a mutual information cost function and six degrees of freedom. Eight different co-registration possibilities with varying source/target images and weighting masks were calculated as previously described (14). Eight different types of co-registrations with varying source/target images and weighting masks were calculated, and the best one with best visual alignment between MRI and PET was chosen as previously described (14). This choice was applied to the average PET image and all PET frames. After transformation, images were resliced with FLIRT using trilinear interpolation and results were verified by visual inspection.

An automated ROI was obtained for the reference region, the cerebellar gray matter. The algorithm assigns probabilistic ROIs to an MRI using an automated multi-label approach (12). 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.

Secondary PET outcome measures: Voxel-wise images of the binding potential BP_P and of the tracer total distribution volume (V_T) (15) were computed for those subjects with usable single venous sample (~65% of the subjects) to confirm main results were consistent with findings when using the primary PET outcome measure. To calculate V_T and BP_P , an input function was obtained using ROI data and simultaneous estimation of

input function with the single venous blood sample as the anchor, as previously validated in (16,17). The anchor was obtained by multiplying the tracer's total radioactivity level in plasma, measured in each subject's blood sample, by an average parent fraction level derived from previously published data with the same tracer (12). This simultaneously estimated input function was then used as the input function at the voxel level to quantify V_T (and from there, $BP_P = V_T - V_{T, ref}$, with $V_{T, ref}$ the average V_T among the voxels in the reference region) in each voxel using Empirical Bayesian Estimation in Graphical Analysis (EBEGA), as described in (18).

Voxel-wise multivariate statistical learning analyses: Multivoxel pattern analysis (MVPA) analyzed the joint BP_{ND} signal across multiple regions in a single subject in order to predict the diagnostic class of that subject. To make MVPA computationally tractable and reduce dimensionality, PET binding maps were resampled from $2 \times 2 \times 2$ mm voxel resolution to $6 \times 6 \times 6$ mm resolution. For all binary classification tasks, a linear kernel Support Vector Machine SVM (19) with a filter feature selection (t -test) and leave-one-out cross-validation was applied using the Spider v1.71 MATLAB toolbox (<http://people.kyb.tuebingen.mpg.de/spider/>) with default regularization parameter $C = 1$. Nuisance effects (sex and site in the case of the FAMPATH and NODA and NODA datasetss) were removed from features (voxel BP_{ND} values) prior to MVPA. During each iteration of leave-one-out cross-validation, one subject was withheld from the data set and: (a) a 2-sample t -test was performed over the remaining training data; (b) the features were ranked by absolute t -score and the top 10, 20, 30...to 400 features were selected; and (c) these selected features were then used to predict the class of the withheld test examples during the classification stage. Classification performance is

reported in terms of “area under the curve” (AUC), i.e., area under the receiver operator characteristic (ROC) curve (20), which is the arithmetic mean of sensitivity (true positive rate) and specificity (true negative rate). Significance was assessed using permutation as in (21). For each top N selected features, 200 permutations were used to generate a null distribution. This approach (plotting performance vs. number of selected features) is unbiased, since for each top selected number of features, and for each round of leave-one-out cross validation, the features were selected from a training set (i.e., total number of samples minus one) that was independent from the testing set (i.e., one left out sample). A corrected p-value that combined the separate p-values (1-tailed) obtained across the top N selected features was computed using Empirical Brown’s Method for combining dependent p-values (22). An additional 1,000 permutations were run using the top N features with the peak classification performance and when testing the model learned in FAMPATH in the second independent dataset (CONTE and NODA studies).

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