Serotonin Transporter Binding in Major Depressive Disorder: Impact of Serotonin System Anatomy

Running Title: Serotonin Transporter System in Major Depression

Elizabeth A Bartlett, PhD^{1,2}, Francesca Zanderigo, PhD^{1,2}, Denise Shieh, MS^{2,4}, Jeffrey Miller, MD^{1,2}, Patrick Hurley, MD^{1,2}, Harry Rubin-Falcone, MS¹, Maria A Oquendo, MD, PhD³, M Elizabeth Sublette, MD, PhD^{1,2}, R Todd Ogden, PhD^{1,2,4}, J John Mann, MD^{1,2,5}

1. *Molecular Imaging and Neuropathology Area, New York State Psychiatric Institute, New York, USA*
2. *Department of Psychiatry, Columbia University Medical Center, New York, USA*
3. *Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
4. *Department of Biostatistics, Mailman School of Public Health, Columbia University Medical Center, New York, USA*
5. *Department of Radiology, Columbia University Medical Center, New York, USA*

***Corresponding Author:** Elizabeth A Bartlett, PhD; eab2266@cumc.columbia.edu; 1051 Riverside Dr, New York, NY 10031

ABSTRACT

Serotonin transporter (5-HTT) binding deficits are reported in major depressive disorder (MDD). However, most studies have not considered serotonin system anatomy when parcellating brain regions of interest (ROIs). We now investigate 5-HTT binding in MDD in two novel ways: (1) use of a 5-HTT tract-based analysis examining binding along serotonergic axons; and (2) using the Copenhagen University Hospital Neurobiology Research Unit (NRU) 5-HT Atlas, based on brain-wide binding patterns of multiple serotonin receptor types. [¹¹C]DASB 5-HTT PET scans were obtained in 59 unmedicated participants with MDD in a current depressive episode and 32 healthy volunteers (HVs). Binding potential (BP_P) was quantified with empirical Bayesian estimation in graphical analysis (EBEGA). Within the [¹¹C]DASB tract, MDD showed significantly lower BP_P compared with HVs ($p=0.02$). The BP_P diagnosis difference varied by tract location at a trend-level ($p=0.08$), with MDD binding deficit strongest most proximal to brainstem raphe nuclei. NRU 5-HT Atlas ROIs showed trend-level lower BP_P in MDD relative to HVs ($p=0.06$) and BP_P diagnosis difference that varied by region ($p=0.001$). BP_P was lower in MDD in 4/10 regions (p -values <0.05). Neither [¹¹C]DASB tract or NRU 5-HT Atlas BP_P correlated with depression severity, suicidal ideation or suicide attempt history. Future studies are needed to determine the causes of this deficit in 5-HTT binding being more pronounced in proximal axon segments and in only a subset of ROIs for the pathogenesis of MDD. Such regional specificity may have implications for targeting antidepressant treatment, and may extend to other serotonin-related disorders.

Keywords: Serotonin transporter, major depressive disorder, positron emission tomography, binding patterns

INTRODUCTION

Altered serotonin (5-HT) transmission has been implicated in major depressive disorder (MDD). Factors that determine serotonin signaling include serotonin neuron firing and release, receptor subtype density and affinity, and removal of serotonin from the synaptic cleft by serotonin transporters (5-HTT). Our knowledge concerning serotonin transporter density and affinity in the brain in MDD is primarily derived from postmortem work and *in vivo* imaging¹. Most postmortem studies of 5-HTT binding in the brain report fewer transporters in MDD, but some studies find no difference in 5-HTT binding, in MDD relative to healthy volunteers (HVs)^{2, 3}. Similarly, most human *in vivo* imaging studies find lower⁴⁻¹³ or similar¹⁴⁻¹⁷ 5-HTT binding in MDD compared with HVs. However, imaging studies typically do not consider serotonergic system anatomy in choosing regions of interest (ROIs), meaning that ROIs may have heterogeneous levels of binding that degrade the binding signal obtained.

Two main anatomic circuits involved in mood regulation have been proposed, the limbic-thalamic-cortical and limbic-striatal-pallidal-thalamic-cortical circuits¹⁸. Our group has previously reported lower 5-HTT binding potential in MDD (BP_p; the ratio of specifically bound radiotracer in tissue to parent radiotracer in plasma¹⁹) in six magnetic resonance imaging (MRI)-defined ROIs (midbrain, putamen, amygdala, thalamus, hippocampus, and anterior cingulate)²⁰, but most recently our group has found no deficit in 5-HTT in MDD (for all binding potential outcome measures) in these same regions¹⁶. PET studies of the serotonin system have generally selected ROIs because they have been implicated in the pathophysiology of MDD and because they had sufficient 5-HTT binding for reliable PET quantification^{20,13}. Moreover, traditional region definitions, e.g., Brodmann Areas, do not conform to serotonin system anatomy. Therefore, these ROIs can contain heterogeneous distributions of serotonin receptors and the transporter, potentially adding noise to data from regions with mixtures of binding levels, thereby creating the potential to miss serotonin-specific pathogenesis.

To identify serotonin-specific dysfunction in MDD, we analyzed [¹¹C]DASB PET 5-HTT binding potential²¹ in two ways in 59 unmedicated patients with MDD in a current depressive episode and 32 HVs. We examined: 1. [¹¹C]DASB binding potential along the general serotonin axonal tract originating in raphe nuclei serotonin neuron cell bodies, projecting upward to the base of the brain, forward to the frontal pole, and then

sweeping back to the occipital pole; and 2. Using the Copenhagen University Hospital Neurobiology Research Unit (NRU) 5-HT Atlas, a set of brain-wide regions based on binding patterns of multiple serotonin targets in the human brain^{22, 23}. Our hypothesis was that the lower [¹¹C]DASB binding potential in unmedicated MDD compared with HVs would show a pattern of difference related to serotonin system anatomy that would not be detectable using general Brodmann Area-related ROIs^{24, 25}. In the MDD group, we also examined relationships of 5-HTT binding potential with depression severity, suicide attempt history and suicidal ideation^{2, 26, 27}.

METHODS

Participants

The data from currently depressed participants with MDD (n=59) and HVs (n=32) in this analysis were leveraged from previously published studies^{16, 28, 29}. As described^{16, 28, 29}, written informed consent was obtained and the Institutional Review Board of the New York State Psychiatric Institute and Columbia University Medical Center approved the study.

MDD participant inclusion criteria included: 1) current major depressive episode; 2) 17-item Hamilton Depression Rating Scale (HDRS) score ≥ 16 at screening; 3) 18–65 years; 4) off all psychotropic and other types of drugs likely to interact with 5-HTT for ≥ 14 days at time of scan. HV inclusion criteria included: 1) absence of current or past DSM-IV Axis I diagnosis, with the exception of specific phobia, 2) absence of cluster B personality diagnosis as assessed using the SCID-II³⁰, 3) 18–65 years. MDD and HV inclusion/exclusion criteria details are listed in Supplemental Information.

The Beck Depression Inventory (BDI)³¹ and HDRS-17³² were used to assess depression severity. The Columbia Suicide History Form³³ and the Scale of Suicidal Ideation³⁴ were used to assess suicide attempt history suicidal ideation, respectively.

PET Acquisition, Processing and Quantification

Details are provided in Supplemental Information and summarized here. [¹¹C]DASB was synthesized and scans were conducted as previously described on an ECAT HR+ scanner (Siemens/CTI, Knoxville, TN, USA))^{35,28}. A polyurethane head holder system (Soule Medical, Tampa, FL, USA) was molded around each

participant's head for immobilization purposes. Each subject received a bolus of radiotracer over 30 seconds (16.16 ± 2.31 mCi) and underwent a 10-minute transmission scan, followed by 100-minute emission scan binned into 19 frames of increasing duration. Images were reconstructed via filtered back projection with a 128×128 matrix²⁸. For arterial input function determination, arterial sampling was performed (automated every 10 seconds for the first 2 minutes and manually at increasing durations thereafter) to obtain arterial plasma radioactivity measurements throughout the scan and unmetabolized parent fractions at six discrete points (2, 12, 20, 50, 80, and 100 minutes via high-performance liquid chromatography)²⁸.

Validated pre-processing was performed, including motion-correction (via registration to the eighth frame using the FMRIB linear image registration tool (FLIRT), version 5.0 (FMRIB Image Analysis Group, Oxford, UK)) and coregistration to subject T1-weighted MRIs with FLIRT^{28,36}. A metabolite-corrected arterial plasma input function was created as described²⁸, and was inputted into empirical Bayesian estimation in graphical analysis (EBEGA)³⁷, a quantification approach designed to handle noise found in PET time activity curves at the voxel level³⁷, to obtain whole-brain, voxel-wise tracer volume of distribution (V_T) maps. Binding potential BP_D maps were then created by subtracting brain-wide estimates of the radiotracer non-displaceable distribution volume (V_{ND}), obtained in each participant using the hybrid deconvolution approach (HYDECA)³⁸, without having to assume any reference region.

Definition of the Serotonin Axonal Tract

At the group level, we observed that [¹¹C]DASB binding maps showed an arc of high binding that tracked closely with the medial serotonergic axon pathway projecting from the cell bodies in the brainstem raphe nuclei into the base of the brain, forwards to the frontal pole via the medial forebrain bundle³⁹, and sweeping back over the cingulate to occipital cortex⁴⁰ (Figure 1, top). Therefore, given this observed pattern of high binding overlapped with known serotonergic system anatomy⁴¹, and the fact that depression-related pathophysiologic changes of depression tend to occur in anterior brain structures¹⁸, we sought to generate a PET-derived ROI to analyze the pattern of 5-HTT binding along this axon tract.

To generate the tract, participant MRI-derived transforms were applied to warp the V_T maps to Montreal Neurological Institute (MNI) space. The warped images were averaged across participants (Figure 1, top) and thresholded at $V_T=11.5$, a value chosen empirically to include the observed high binding areas overlapping with the core medial serotonergic pathway. To examine the core trajectory of serotonin axons projecting from the raphe nuclei to the occipital cortex, the medial 20 voxels were selected. Projections to regions adjacent to the axon tract like thalamus, basal ganglia, cerebellum and the cortical mantle were excluded because our focus was on the core medial axon tract⁴⁰. Although axons fan out from this tract to subcortical areas and the entire cortical mantle, we sought to sample from origin to terminus, such that each axon tract segment is more distal than its previous adjacent segment (Figure 1). The resulting serotonin axonal tract was then binarized to create a single ROI (Figure 1, middle).

To analyze the pattern of 5-HTT binding potential along the tract, the binary tract was parcellated as follows (see flowchart in Supplemental Figure 1). The center sagittal slice was isolated and voxels on the border of the tract were retained. Starting at the origin in the raphe nuclei, a line was drawn through the first voxel to connect it with the voxels on the border directly surrounding it. Then, a line normal to that line was projected through the center of the tract to the opposite border, which created the dividing line between the first and second parcel. To ensure adequate estimation of binding potential in each parcel along the tract, we set the minimum volume to 100 voxels. If there were ≥ 100 voxels when the parcel was extended from the center sagittal slice to all slices in the tract, the parcel was finalized. If there were < 100 voxels, the next voxel on the tract border was considered and the line-drawing procedure (steps 4a & 4b, Supplemental Figure 1) was repeated for the parcel at hand. This was iterated until all parcels were finalized through the occipital cortex terminus. This resulted in 56 parcels, 100-400 voxels in size (Figure 1, bottom).

Serotonin Axonal Tract Statistics

To test our *a priori* hypothesis of lower 5-HTT BP_P in MDD that varies as a function of axonal tract location, we first analyzed the entire tract as a single ROI (i.e., participant-wise average BP_P across all voxels in the tract) and compared diagnoses with t-test and linear regression (also considering age and sex).

Next, we computed BP_P within each parcel along the tract, considering the observations along the tract as (nearly) continuous and applying principles of functional data analysis⁴². The estimated 5-HTT BP_P pattern for each participant was represented as a function of distance along the tract. Using these participant-level estimates, the mean difference between MDD and HV groups was estimated at each point along the tract. To test for a main effect (consistent along the tract) of diagnostic group, the test statistic was the absolute value of the integrated difference of group mean functions. The significance of this measure was assessed based on 1,000 random permutations of group labels. To determine whether effect of diagnostic group was constant along the tract (a “pure” main effect) or if it varied as a function of location along the tract, equivalent to a test for interaction between diagnosis and tract location, the test statistic was the integrated squared difference between the two mean

functions, after adjusting for the estimated main effect. Significance was based on 1,000 random permutations of group labels. The above analyses were then repeated controlling for both age and sex, and repeated only within the MDD group to explore relationships with depression severity (HDRS-17 and BDI), suicide attempt history, and history of suicidal ideation.

NRU 5-HT Atlas Processing

To obtain ROIs with uniform 5-HTT BP_P, we used the NRU 5-HT Atlas (Supplemental Figure 1 (center) and Figure 4 (bottom); <https://xtra.nru.dk/FS5ht-atlas/>)^{22, 23}. This atlas, created by clustering maps of PET binding to 5-HTT and 5-HT 1A, 1B, 2A, and 4 receptors, offers 10 brain regions with homogeneous distributions of serotonin system components²³. The NRU 5-HT Atlas is provided as FreeSurfer *fsaverage* overlays^{22, 23}. Each participant's BP_P map in individual MR-space was resampled onto the *fsaverage* surface using FreeSurfer 7.1.1 reconstruction stream (<http://surfer.nmr.mgh.harvard.edu/>) run for their T1-weighted MRIs. Average BP_P was extracted for the 10 serotonin regions (excluding negative values).

NRU 5-HT Atlas Statistics

To borrow strength across NRU 5-HT Atlas ROIs and account for correlation among ROIs, we fit linear mixed-effects (LME) models to the ROI-level BP_P estimates after logarithmic transformation, with participant as a random effect. The statistics for the NRU 5-HT Atlas paralleled that of the serotonin axonal tract. We first tested for a main effect of diagnosis i.e., a group effect that is consistent across regions. Secondly, we tested for a region-by-diagnosis interaction effect. The above modeling strategy was repeated while controlling for sex and age and repeated to explore effects with depression severity, suicide attempt history, and suicidal ideation in the MDD group. Statistics were performed in R v4.0.3^{43, 44}.

RESULTS

Participants

Table 1 displays demographics and imaging characteristics. At enrollment, of participants with MDD, 23.7% were antidepressant medication (AM) naïve, 5.1% had unknown AM exposure, 39.0% were off AM ≥ 12 weeks, and 32.2% were off AM <12 weeks (with 9 participants reporting AM exposure within 2 weeks and

therefore requiring washout prior to scanning). Participants with MDD were older than HVs and were moderately depressed at enrollment. [¹¹C]DASB injected dose did not differ by diagnosis.

Table 1: MDD and Healthy Volunteer Participant Characteristics			
	MDD (n=59)	HV (n=32)	p-value
Age (yrs.)	39.6 ± 11.3	32.8 ± 11.1	p=0.007
% Female	52.5%	53.1%	p=1
% Hispanic	20.3%	9.4%	p=0.25
% Race	74.6% White 6.8% Asian 11.9% Black 5.1% More than one race 1.7% American Indian/Alaskan Native	50.0% White 18.8% Asian 18.8% Black 3.1% More than one race 9.4% unknown/not reported	p=0.03
Years Education	15.6 ± 2.6	16.1 ± 1.9	p=0.26
BDI	22.7 ± 10.0	1.8 ± 3.0	p<0.001
HDRS-17	18.2 ± 4.4	1.6 ± 3.0	p<0.001
SSI Total Score	5.5 ± 6.7	0 ± 0	p<0.001
% Suicide Attempt History*	30.5%	0%	p<0.001
[¹¹C]DASB Injected Dose (mCi)	16.2 ± 2.3	16.2 ± 2.4	p=0.97
Abbreviations: BDI = Beck Depression Inventory ³¹ , HDRS-17 = Hamilton Depression Rating Scale 17-item ⁴⁵ , SSI = Scale for Suicidal Ideation ³⁴ ; data missing from 14 participants, MDD=major depressive disorder, HV=healthy volunteer			

5-HTT Binding Potential in the Serotonin Axon Tract

Average 5-HTT [¹¹C]DASB BP_P across the entire non-parcellated serotonin axonal tract (Figure 1, middle), was 13.5% lower in MDD compared with HVs ($p<0.001$). When considering the pattern of BP_P along the parcellated tract (Figure 1, bottom), a main effect of diagnosis was found ($p=0.022$). As shown in Figures 2 and 3, for most locations along the tract, depressed individuals had lower 5-HTT BP_P compared to HVs. Figure 3 displays permutation testing results, where 95% pointwise confidence intervals show that the diagnostic group difference in 5-HTT BP_P excluded a zero estimate from the origin in the raphe nuclei through the first ~25% of the tract, approximately at the anterior cingulate cortex. The tract closest to the origin of serotonergic axons had the largest deficit of 5-HTT BP_P in MDD. The location with the largest diagnosis difference (Figure 3) was within the ventral tegmental area (VTA) of the midbrain, where MDD 5-HTT BP_P was 18.1% lower than HVs

(estimate=-4.77). Near the occipital lobe terminus, the 5-HTT BP_P group difference reached zero. A robust diagnosis difference persisted until the genu of the anterior cingulate, and then abruptly declined to a small to absent group difference thereafter until the occipital lobe terminus (Figure 3).

The pattern of 5-HTT BP_P group difference differed from a constant function at a trend-level as a function of distance from axonal origin ($p=0.076$, Supplemental Figure 2). When sex and age were included, the above effects persisted (main effect of diagnosis: $p=0.016$ and diagnosis by location interaction: $p=0.068$).

In exploratory analyses within the MDD group, 5-HTT BP_P in the tract did not correlate with depression severity (HDRS-17: $p=0.91$ or BDI: $p=0.58$), suicide attempt history ($p=0.60$), or suicidal ideation ($p=0.79$) in separate models for each variable.

NRU 5-HT Atlas

An example of one participant's $[^{11}\text{C}]\text{DASB}$ BP_p map, the NRU 5-HT Atlas parcellation, and the $[^{11}\text{C}]\text{DASB}$ tract sampled onto the FreeSurfer *fsaverage* surface are shown in Supplemental Figure 3 for comparison.

Across all NRU 5-HT Atlas regions, although we found a trend-level main effect of lower BP_p in the MDD diagnostic group ($F=3.52$, $p=0.06$), when we examined the effects of brain region, we found a diagnosis-by-region interaction ($F=2.91$, $p=0.001$). Post-hoc testing by region, indicated lower 5-HTT binding potential in MDD compared with HVs in all 10 NRU 5-HT regions (Supplemental Table 2, Figure 4; indicated by negative beta estimates (range -0.08 to -0.16)). The strongest effects of lower BP_p in MDD were in region 2 (95% Confidence Interval (CI)=[-0.27, -0.01], $p=0.03$), including portions of the supramarginal, superior and transverse temporal, and insular cortices, region 3 (95% CI=[-0.29, -0.03], $p=0.02$), including portions of the precentral, postcentral, and paracentral cortices, region 6 (95% CI=[-0.27, -0.005], $p=0.04$), including portions of the superior

temporal, temporal pole, entorhinal, parahippocampal, insular, rACC, and medial and lateral orbitofrontal cortices, and region 7 (95% CI=[-0.29, -0.03], $p=0.02$), including portions of the isthmus cingulate, parahippocampal, and entorhinal cortices (Supplemental Table 2; Figure 4). When sex and age were included, the above effects persisted (main effect: $p=0.06$ and interaction with region: $p=0.002$).

In exploratory analyses within the MDD group, there were no significant relationships between BP_p and depression severity (HDRS-17 or BDI), suicide attempt history, or suicidal ideation (all p -values >0.05).

Figure 4: Displaying the estimated lower 5-HTT binding potential in MDD relative to the HV group found within the NRU 5-HT Atlas, where 5-HTT binding potential (BP_p) is plotted for all regions in the NRU 5-HT Atlas, broken down by diagnosis (HV=healthy volunteer with circles, MDD = major depressive disorder with triangles) (top). Regions with $p < 0.05$ in post hoc analysis denoted with * and p-value is shown. The color-coding for each region in the top graph matches the corresponding region in the NRU 5-HT Atlas shown at bottom with labeling for region number.

DISCUSSION

Within the serotonin axonal tract, there was an MDD [^{11}C]DASB BP_P deficit compared with HVs ($p=0.02$) and a trend-level effect of MDD deficit by tract length ($p=0.08$). The MDD 5-HTT deficit was strongest in the tract most proximal to the serotonin neuron cell bodies in the midbrain raphe nuclei, persisting until the genu of the anterior cingulate, and rapidly dissipating through to the tract terminus in occipital pole. We found a region-wise effect ($p=0.001$). 5-HTT BP_P was lower in 4/10 regions of distinct serotonergic characteristics ($p=0.02-0.04$). We did not find that these deficits correlated with depression severity, suicidal ideation, or suicide attempt history.

[^{11}C]DASB Tract Analysis

Consistent with prior findings of ~10% less 5-HTT binding in many brain regions in depression², we found 13.5% lower 5-HTT [^{11}C]DASB BP_P averaged over the entire serotonin axonal tract in MDD. When we considered BP_P in each parcel along the tract, as seen in Figure 3, the MDD BP_P deficits were greatest toward the proximal section of the tract until the genu of anterior cingulate and dissipated thereafter. No other such study has been published and replication of this finding is important, as is seeking an explanation of its cause. Perhaps axon transportation impairment or regulation of 5-HTT levels by low intra-synaptic serotonin levels are more pronounced in more proximal parts of serotonin projections.

A cause of lower 5-HTT binding in MDD may include gene variants such as 5-HTTLPR in the upstream regulatory region, some of whose alleles associate with less 5-HTT mRNA expression *in vitro*^{45, 46}, including specifically in depressed suicide decedents⁴⁷, but with mixed findings *in vivo*^{16, 48-51}. Such a genomic effect in rodents was confined to the brainstem and not seen in the hippocampus⁵². A second related mechanism is epigenetic modification and DNA methylation effects from early adversity reducing 5-HTT binding in adulthood⁵³. Another mechanism for deficits in 5-HTT binding is accelerated transporter internalization at the synaptic level due to less serotonin release from upregulation of 5-HT_{1A} autoreceptors in depressed suicidal individuals⁵⁴. This mechanism may be region-specific. Such 5-HTT internalization due to less serotonin release may be considered compensatory instead of causal in the pathogenesis of MDD. Finally, axonal transport of

mRNA or proteins from the cell body, as exemplified by the transporter deficit, may be abnormal in MDD. The mechanisms responsible for these findings have important treatment implications.

The greatest MDD 5-HTT BP_P deficit encompasses the VTA, which is the origin of the mesolimbic dopamine system, mediating reward, motivation, and adaptive behaviors^{55, 56, 57}. The VTA dopamine neurons are richly innervated by afferent serotonin neurons originating in raphe nuclei⁵⁸⁻⁶⁰, that exert excitatory control. Conditioned place preference in rodents, a paradigm reflecting motivation and reward, was induced, and dopamine released in the nucleus accumbens by VTA activation of 5-HT neurons was subsequently blocked by VTA inactivation of serotonin and glutamate receptors⁶¹. Lower 5-HTT binding in MDD can have effects on limbic and cortical efferent systems through excitatory and inhibitory interactions with multiple neurotransmitter systems⁶⁰.

NRU 5-HT Atlas Analysis

Leveraging serotonergic system anatomy to define ROIs on the basis of more uniform serotonin component levels^{22, 23}, we found a significant interaction of diagnosis-by-region 5-HTT binding potential.

Four of ten regions exhibited 5-HTT BP_P deficits in MDD with 95% CIs that excluded a zero estimate (p-values=0.02-0.04). Interestingly, these regions do not overlap with the serotonin axonal tract; NRU 5-HT Atlas region 8 shows the greatest overlap with the tract (Supplementary Figure 1); however, does not include the brainstem and medial forebrain bundle areas where MDD 5-HTT deficits were greatest in the tract analysis, potentially explaining why region 8 did not reach post-hoc significance for MDD deficits. Given the relative widespread nature of the four regions with 5-HTT deficit in MDD, it is difficult to posit a neuroanatomical hypothesis for their involvement in depression pathophysiology. Meta-analyses suggest a widespread pattern of 5-HTT deficiency in depression (e.g., in the brainstem, amygdala, hippocampus, thalamus, striatum, frontal cortex, and cingulate cortex^{2, 3}). We previously used six Brodmann area-based ROIs overlapping with these regions, selected for their history in depression pathophysiology and based on them having relatively high 5-HTT PET binding, offering a more robust measurement compared with low binding regions¹⁶. In those six Brodmann regions, we found no difference in 5-HTT binding (outcome measures: V_T/f_P, BP_P, BP_{ND}, BP_F) in MDD relative to HV¹⁶, which disagreed with previous reports of low 5-HTT binding in MDD⁴⁻¹³. Standard ROIs mean a mismatch

with serotonin binding patterns or heterogenous binding levels, and that weakens the ability to detect disease effects. The serotonin atlas-based ROIs showed how regional binding differed and there was a robust region-dependent 5-HTT binding deficit in MDD. Future studies should consider a potential advantage in considering anatomical binding pattern of a neurotransmitter-specific imaging agent in analyzing brain imaging data.

Strengths, Limitations, and Future Directions

Strengths of this study include a large sample size for an *in vivo* PET study, employing full quantification approach with an input function and ROIs based on serotonin system anatomy. A strength of the axonal tract analysis is that it only required two statistical tests to determine if a diagnosis effect was present and if it varied as a function of tract location, in contrast to voxel-level analyses employing thousands of tests and complex multiple comparisons correction techniques. There are also limitations. The high-binding V_T threshold used to generate the serotonin axonal tract was selected empirically based on binding level. This threshold could be further optimized and estimated in a data-driven manner in future work, which could even include individualized thresholding to obtain tracts that better conform to individual-level serotonin system anatomy.

Conclusion

Understanding the mechanisms by which the serotonin transporter plays a role in major depressive disorder onset and progression can assist prevention and treatment development. We found evidence for 5-HTT deficits in MDD in a midline serotonin axonal tract and region-specific serotonergic pathology. Such serotonin-specific anatomical specificity may offer new treatment targets in MDD.

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REFERENCES

1. Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clin Chem* 1994; **40**(2): 288-295.
2. Gryglewski G, Lanzenberger R, Kranz GS, Cumming P. Meta-analysis of molecular imaging of serotonin transporters in major depression. *Journal of Cerebral Blood Flow & Metabolism* 2014; **34**(7): 1096-1103.
3. Kambeitz JP, Howes OD. The serotonin transporter in depression: Meta-analysis of in vivo and post mortem findings and implications for understanding and treating depression. *Journal of affective disorders* 2015; **186**: 358-366.
4. Parsey RV, Hastings RS, Oquendo MA, Huang Y-y, Simpson N, Arcement J *et al.* Lower serotonin transporter binding potential in the human brain during major depressive episodes. *American Journal of Psychiatry* 2006; **163**(1): 52-58.
5. Selvaraj S, Murthy NV, Bhagwagar Z, Bose SK, Hinz R, Grasby PM *et al.* Diminished brain 5-HT transporter binding in major depression: a positron emission tomography study with [11 C] DASB. *Psychopharmacology* 2011; **213**(2-3): 555-562.
6. Malison RT, Price LH, Berman R, Van Dyck CH, Pelton GH, Carpenter L *et al.* Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 β -carbomethoxy-3 β -(4-iodophenyl) tropane and single photon emission computed tomography. *Biological psychiatry* 1998; **44**(11): 1090-1098.
7. Joensuu M, Tolmunen T, Saarinen PI, Tiihonen J, Kuikka J, Ahola P *et al.* Reduced midbrain serotonin transporter availability in drug-naive patients with depression measured by SERT-specific [123I] nor- β -CIT SPECT imaging. *Psychiatry Research: Neuroimaging* 2007; **154**(2): 125-131.
8. Reimold M, Batra A, Knobel A, Smolka M, Zimmer A, Mann K *et al.* Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [11 C] DASB PET study. *Molecular psychiatry* 2008; **13**(6): 606-613.
9. Staley JK, Sanacora G, Tamagnan G, Maciejewski PK, Malison RT, Berman RM *et al.* Sex differences in diencephalon serotonin transporter availability in major depression. *Biological psychiatry* 2006; **59**(1): 40-47.

10. Willeit M, Praschak-Rieder N, Neumeister A, Pirker W, Asenbaum S, Vitouch O *et al.* [123I]- β -CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. *Biological psychiatry* 2000; **47**(6): 482-489.
11. Lehto S, Tolmunen T, Joensuu M, Saarinen PI, Vanninen R, Ahola P *et al.* Midbrain binding of [123I] nor- β -CIT in atypical depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2006; **30**(7): 1251-1255.
12. Nye JA, Purselle D, Plisson C, Voll RJ, Stehouwer JS, Votaw JR *et al.* Decreased brainstem and putamen SERT binding potential in depressed suicide attempters using [11C] - ZIENT pet imaging. *Depression and anxiety* 2013; **30**(10): 902-907.
13. Cannon DM, Ichise M, Fromm SJ, Nugent AC, Rollis D, Gandhi SK *et al.* Serotonin transporter binding in bipolar disorder assessed using [11C] DASB and positron emission tomography. *Biological psychiatry* 2006; **60**(3): 207-217.
14. Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K *et al.* Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [11C] DASB PET imaging study. *American Journal of Psychiatry* 2001; **158**(11): 1843-1849.
15. Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N *et al.* Brain serotonin transporter binding potential measured with carbon11-labeled dasb positron emission tomography: Effects of major depressive episodes and severity of dysfunctional attitudes. *Archives of general psychiatry* 2004; **61**(12): 1271-1279.
16. Miller JM, Hesselgrave N, Ogden RT, Sullivan GM, Oquendo MA, Mann JJ *et al.* Positron emission tomography quantification of serotonin transporter in suicide attempters with major depressive disorder. *Biological psychiatry* 2013; **74**(4): 287-295.
17. Ichimiya T, Suhara T, Sudo Y, Okubo Y, Nakayama K, Nankai M *et al.* Serotonin transporter binding in patients with mood disorders: a PET study with [11C](+) McN5652. *Biological Psychiatry* 2002; **51**(9): 715-722.
18. Soares JC, Mann JJ. The functional neuroanatomy of mood disorders. *Journal of psychiatric research* 1997; **31**(4): 393-432.
19. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN *et al.* Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *Journal of Cerebral Blood Flow & Metabolism* 2007; **27**(9): 1533-1539.

20. Parsey RV, Hastings RS, Oquendo MA, Huang YY, Simpson N, Arcement J *et al.* Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am J Psychiatry* 2006; **163**(1): 52-58.
21. Frankle WG, Huang Y, Hwang D-R, Talbot PS, Slifstein M, Van Heertum R *et al.* Comparative Evaluation of Serotonin Transporter Radioligands 11C-DASB and 11C-McN 5652 in Healthy Humans. *J Nucl Med* 2004; **45**(4): 682-694.
22. Beliveau V, Ozenne B, Strother S, Greve DN, Svarer C, Knudsen GM *et al.* The structure of the serotonin system: A PET imaging study. *NeuroImage* 2020; **205**: 116240.
23. Beliveau V, Ganz M, Feng L, Ozenne B, Højgaard L, Fisher PM *et al.* A high-resolution in vivo atlas of the human brain's serotonin system. *Journal of Neuroscience* 2017; **37**(1): 120-128.
24. Gryglewski G, Lanzenberger R, Kranz GS, Cumming P. Meta-analysis of molecular imaging of serotonin transporters in major depression. *J Cereb Blood Flow Metab* 2014; **34**(7): 1096-1103.
25. Kambeitz JP, Howes OD. The serotonin transporter in depression: Meta-analysis of in vivo and post mortem findings and implications for understanding and treating depression. *J Affect Disord* 2015; **186**: 358-366.
26. Gopaldas M, Zanderigo F, Zhan S, Ogden RT, Miller JM, Rubin-Falcone H *et al.* Brain serotonin transporter binding, plasma arachidonic acid and depression severity: a positron emission tomography study of major depression. *Journal of affective disorders* 2019; **257**: 495-503.
27. Yeh Y-W, Ho P-S, Chen C-Y, Kuo S-C, Liang C-S, Yen C-H *et al.* Suicidal ideation modulates the reduction in serotonin transporter availability in male military conscripts with major depression: a 4-[18F]-ADAM PET study. *The World Journal of Biological Psychiatry* 2015; **16**(7): 502-512.
28. Ogden RT, Ojha A, Erlandsson K, Oquendo MA, Mann JJ, Parsey RV. In vivo quantification of serotonin transporters using [(11)C]DASB and positron emission tomography in humans: modeling considerations. *J Cereb Blood Flow Metab* 2007; **27**(1): 205-217.

29. First M, Spitzer R, Gibbon M, Williams J. *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P, Version 2.0)*. Biometrics Research Dept., New York State Psychiatric Institute: New York, 1995.
30. First MB, Gibbon M, Spitzer RL, Williams JB, Benjamin LS. *SCID-II Personality Questionnaire*. American Psychiatric Press: Washington, D.C., 1997.
31. Beck AT, Ward CH, Mendelson M, Mock J, Erbauh J. An inventory for measuring depression. *Arch Gen Psychiatry* 1961; **4**: 53-63.
32. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psych* 1960; **23**: 56-62.
33. Oquendo MA, Halberstam B, Mann JJ. Risk factors for suicidal behavior: the utility and limitations of research instruments. In: First MB (ed). *Standardized Evaluation in Clinical Practice*. American Psychiatric Publishing: Arlington, VA, 2003, pp 103-130.
34. Beck AT, Kovacs M, Weissman A. Assessment of suicidal intention: the Scale for Suicide Ideation. *Journal of consulting and clinical psychology* 1979; **47**(2): 343.
35. Belanger MJ, Simpson NR, Wang T, Van Heertum R, Mann JJ, Parsey RV. Biodistribution and Radiation Dosimetry of [11C]DASB in Baboons. *Nucl Med Biol* 2004; **31**(8): 1097-1102.
36. Parsey RV, Slifstein M, Hwang DR, Abi-Dargham A, Simpson N, Mawlawi O *et al*. Validation and reproducibility of measurement of 5-HT1A receptor parameters with [carbonyl-11C]WAY-100635 in humans: comparison of arterial and reference tissue input functions. *J Cereb Blood Flow Metab* 2000; **20**(7): 1111-1133.
37. Zanderigo F, Ogden RT, Bertoldo A, Cobelli C, Mann JJ, Parsey RV. Empirical Bayesian estimation in graphical analysis: a voxel-based approach for the determination of the volume of distribution in PET studies. *Nuclear medicine and biology* 2010; **37**(4): 443-451.
38. Zanderigo F, Mann JJ, Ogden RT. A hybrid deconvolution approach for estimation of in vivo non-displaceable binding for brain PET targets without a reference region. *PloS one* 2017; **12**(5): e0176636.
39. Hornung J-P. The neuronatomy of the serotonergic system. *Handbook of Behavioral Neuroscience*, vol. 21. Elsevier 2010, pp 51-64.

40. Charnay Y, Léger L. Brain serotonergic circuitries. *Dialogues in clinical neuroscience* 2010; **12**(4): 471.
41. Törk I. Anatomy of the Serotonergic System a. *Annals of the New York Academy of Sciences* 1990; **600**(1): 9-34.
42. Wang J-L, Chiou J-M, Müller H-G. Functional data analysis. *Annual Review of Statistics and Its Application* 2016; **3**: 257-295.
43. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823* 2014.
44. Team RC. R: A language and environment for statistical computing. Vienna, Austria 2013.
45. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* 1960; **23**: 56-62.
46. Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D *et al.* Allelic variation of human serotonin transporter gene expression. *Journal of neurochemistry* 1996; **66**(6): 2621-2624.
47. Lesch K-P, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**(5292): 1527-1531.
48. Arango V, Underwood MD, Boldrini M, Tamir H, Kassir SA, Hsiung S-c *et al.* Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology* 2001; **25**(6): 892-903.
49. Parsey RV, Hastings RS, Oquendo MA, Hu X, Goldman D, Huang Y-y *et al.* Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. *American Journal of Psychiatry* 2006; **163**(1): 48-51.

50. Güzey C, Allard P, Brännström T, Spigset O. Radioligand binding to brain dopamine and serotonin receptors and transporters in Parkinson's disease: relation to gene polymorphisms. *International Journal of Neuroscience* 2012; **122**(3): 124-132.
51. Reimold M, Smolka M, Schumann G, Zimmer A, Wrase J, Mann K *et al.* Midbrain serotonin transporter binding potential measured with [¹¹C] DASB is affected by serotonin transporter genotype. *Journal of neural transmission* 2007; **114**(5): 635-639.
52. Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, Boovariwala A, Willeit M *et al.* Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [¹¹C] DASB positron emission tomography study. *Biological psychiatry* 2007; **62**(4): 327-331.
53. Albert PR, Benkelfat C. The neurobiology of depression—revisiting the serotonin hypothesis. II. Genetic, epigenetic and clinical studies. The Royal Society 2013.
54. Kinnally EL, Capitanio JP, Leibel R, Deng L, LeDuc C, Haghighi F *et al.* Epigenetic regulation of serotonin transporter expression and behavior in infant rhesus macaques. *Genes, Brain and Behavior* 2010; **9**(6): 575-582.
55. Oquendo MA, Galfalvy H, Sullivan GM, Miller JM, Milak MM, Sublette ME *et al.* Positron emission tomographic imaging of the serotonergic system and prediction of risk and lethality of future suicidal behavior. *JAMA psychiatry* 2016; **73**(10): 1048-1055.
56. Wise RA. Dopamine, learning and motivation. *Nature reviews neuroscience* 2004; **5**(6): 483-494.
57. Kalivas PW, Nakamura M. Neural systems for behavioral activation and reward. *Current opinion in neurobiology* 1999; **9**(2): 223-227.
58. Oades RD, Halliday GM. Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Research Reviews* 1987; **12**(2): 117-165.
59. Hervé D, Pickel VM, Joh TH, Beaudet A. Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. *Brain research* 1987; **435**(1-2): 71-83.

60. Van Bockstaele EJ, Cestari DM, Pickel VM. Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. *Brain research* 1994; **647**(2): 307-322.
61. Neurocircuitry of mood disorders. *Proceedings of the In Neuropsychopharmacology: The Fifth Generation of Progress* 2002. Citeseer.
62. Wang H-L, Zhang S, Qi J, Wang H, Cachope R, Mejias-Aponte CA *et al.* Dorsal raphe dual serotonin-glutamate neurons drive reward by establishing excitatory synapses on VTA mesoaccumbens dopamine neurons. *Cell reports* 2019; **26**(5): 1128-1142. e1127.

Supplemental Information

PET Pre-Processing and Quantification

As previously described, to correct for head motion, PET frames were registered to the eighth frame using the FMRIB linear image registration tool (FLIRT), version 5.0 (FMRIB Image Analysis Group, Oxford, UK). Each subject's mean PET image was coregistered to their T1-weighted MRI (details previously described^{28,37}) with FLIRT, optimized as previously described³⁸.

The arterial input function was generated by taking the product of the unmetabolized parent fraction curve, which was fit using a damped biexponential function, and the plasma total radioactivities curve, and by fitting the product curve with a straight line from time zero to activity peak and a sum of three decreasing exponentials thereafter²⁸. Whole-brain, voxel-wise tracer volume of distribution (V_T) maps were quantified via empirical Bayesian estimation in graphical analysis (EBEGA)³⁹, a quantification approach designed to handle noise found in PET time activity curves at the voxel level³⁹. As our outcome measure of interest was the binding potential BP_P , the V_T maps were then used to create voxel-wise BP_P maps by subtracting in each voxel a brain-wide estimate of the radiotracer non-displaceable distribution volume (V_{ND}), obtained in each individual using the hybrid deconvolution approach (HYDECA)⁴⁰, without having to assume any reference region.

Participant Exclusion Criteria

MDD participant exclusion criteria included: 1) current or past psychotic illness or bipolar disorder, 2) anorexia nervosa or bulimia nervosa in the past year, 3) drug or alcohol abuse within the past two months or dependence within six months, 4) first-degree family history of schizophrenia in subjects <33 years³¹, 5) significant active physical illness, 6) lack capacity to consent to study participation, 7) pregnancy or lactation in women, 8) previous head injury with loss of consciousness for more than three minutes and deficits on testing, and 9) exposure to 3,4- methylenedioxymethamphetamine (MDMA) on more than two occasions.

HV exclusion criteria included MDD exclusion criteria 5-9 above as well as: 1) past or present alcohol/substance abuse or dependence; 2) first-degree relative with history of major depression, schizophrenia, schizoaffective disorder, or suicide attempt; and 3) two or more first-degree relatives with a history of substance dependence.

Supplemental Figure 1: Flowchart of [^{11}C]DASB tract generation.

Supplemental Figure 2: Diagnostic group by distance along the tract interaction test: After controlling for the constant main effect of diagnosis, the difference in 5-HTT BP_D between MDD = major depressive disorder and HV = healthy volunteer groups is shown plotted against the distance along the tract. The group difference in the original dataset is plotted with a thick, solid red line. Permutation testing for the interaction of diagnostic difference in binding by distance along the tract yielded a p-value of 0.076. Black lines show the 95% confidence intervals obtained via bootstrapping. Locations along the tract with both confidence intervals above or below 0 indicate locations of significant group differences.

Supplemental Figure 3: Representative $[^{11}\text{C}]\text{DASB } BP_p$ voxel-wise map resampled to the FreeSurfer fsaverage surface (left) shown in comparison to the NRU 5-HT atlas parcellation (center) and the $[^{11}\text{C}]\text{DASB}$ Tract viewed as a single ROI (right). Left hemisphere shown throughout, with lateral (top) and medial (bottom) surfaces shown.

Supplemental Table 1: NRU 5-HT atlas MDD vs. HV 5-HTT binding difference linear mixed model results by region			
	Beta Estimate	95% Confidence Interval	p-value
Region 1	-0.12	[-0.25, 0.01]	p=0.08
Region 2	-0.14	[-0.27, -0.01]	p=0.03
Region 3	-0.16	[-0.29, -0.02]	p=0.02
Region 4	-0.11	[-0.24, 0.02]	p=0.09
Region 5	-0.08	[-0.21, 0.05]	p=0.22
Region 6	-0.14	[-0.27, -0.005]	p=0.04
Region 7	-0.16	[-0.29, -0.03]	p=0.02
Region 8	-0.11	[-0.24, 0.02]	p=0.10
Region 9	-0.10	[-0.23, 0.03]	p=0.13
Region 10	-0.09	[-0.22, 0.04]	p=0.19
**Results stem from a rejection of the null hypothesis that 5-HTT binding potential is the same across all regions (p=0.001). Standard error for all regions = 0.06, degrees of freedom for all regions = 99.8			