

# Assessment of Striatal Dopamine Transporter Binding in Individuals With Major Depressive Disorder In Vivo Positron Emission Tomography and Postmortem Evidence

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 Supplemental content

**IMPORTANCE** Major depressive disorder (MDD) might involve dopamine (DA) reductions. The DA transporter (DAT) regulates DA clearance and neurotransmission and is sensitive to DA levels, with preclinical studies (including those involving inescapable stressors) showing that DAT density decreases when DA signaling is reduced. Despite preclinical data, evidence of reduced DAT in MDD is inconclusive.

**OBJECTIVE** Using a highly selective DAT positron emission tomography (PET) tracer ( $[^{11}\text{C}]$  altropine), DAT availability was probed in individuals with MDD who were not taking medication. Levels of DAT expression were also evaluated in postmortem tissues from donors with MDD who died by suicide.

**DESIGN, SETTING, AND PARTICIPANTS** This cross-sectional PET study was conducted at McLean Hospital (Belmont, Massachusetts) and Massachusetts General Hospital (Boston) and enrolled consecutive individuals with MDD who were not taking medication and demographically matched healthy controls between January 2012 and March 2014. Brain tissues were obtained from the Douglas-Bell Canada Brain Bank. For the PET component, 25 individuals with current MDD who were not taking medication and 23 healthy controls recruited from McLean Hospital were included (all provided usable data). For the postmortem component, 15 individuals with depression and 14 healthy controls were considered.

**INTERVENTION** PET scan.

**MAIN OUTCOMES AND MEASURES** Striatal and midbrain DAT binding potential was assessed. For the postmortem component, tyrosine hydroxylase and DAT levels were evaluated using Western blots.

**RESULTS** Compared with 23 healthy controls (13 women [56.5%]; mean [SD] age, 26.49 [7.26] years), 25 individuals with MDD (19 women [76.0%]; mean [SD] age, 26.52 [5.92] years) showed significantly lower in vivo DAT availability in the bilateral putamen and ventral tegmental area (Cohen  $d$  range,  $-0.62$  to  $-0.71$ ), and both reductions were exacerbated with increasing numbers of depressive episodes. Unlike healthy controls, the MDD group failed to show an age-associated reduction in striatal DAT availability, with young individuals with MDD being indistinguishable from older healthy controls. Moreover, DAT availability in the ventral tegmental area was lowest in individuals with MDD who reported feeling trapped in stressful circumstances. Lower DAT levels (and tyrosine hydroxylase) in the putamen of MDD compared with healthy controls were replicated in postmortem analyses (Cohen  $d$  range,  $-0.92$  to  $-1.15$ ).

**CONCLUSIONS AND RELEVANCE** Major depressive disorder, particularly with recurring episodes, is characterized by decreased striatal DAT expression, which might reflect a compensatory downregulation due to low DA signaling within mesolimbic pathways.

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**D**espite decades of research, the molecular underpinnings of major depressive disorder (MDD) remain incompletely understood. Among various targets, dopamine (DA) has received considerable attention, particularly owing to its role in motivation, which is affected in MDD.<sup>1</sup> Growing data point to blunted DA transmission in MDD. The most direct evidence stems from DA depletion studies, which have described rapid increases in depressive symptoms after catecholamine depletion in remitted MDD.<sup>2-4</sup> Second, functional magnetic resonance imaging studies have shown that MDD is associated with reduced reward-associated activation within DA-rich regions, including the ventral (nucleus accumbens [NAc]) and dorsal (caudate and putamen) striatum.<sup>5,6</sup> Notably, such blunting could be normalized by a pharmacological challenge hypothesized to transiently increase DA signaling,<sup>7</sup> suggesting that blunted striatal responses might be linked to hypodopaminergic mechanisms. Third, animal models relevant to depression reliably induce anhedonic phenotypes and mesolimbic DA abnormalities.<sup>1,8</sup>

Neurobiologically, these preclinical models trigger a long-lasting downregulation of mesolimbic DA pathways<sup>8</sup> and reduced levels of the DA transporter (DAT).<sup>9</sup> Reduced DAT levels, interpreted as reflecting compensatory DAT downregulation owing to blunted DA transmission, have been reported in the NAc, caudate, or putamen of animals exposed to chronic stressors.<sup>9-11</sup> Reduced striatal DAT levels have been also described in rats bred for increased vulnerability to depression.<sup>12</sup>

The DAT, which is localized on the membrane of presynaptic terminals, plays a central role in regulating the intensity and duration of dopaminergic transmission in the synaptic cleft by reuptaking DA into presynaptic cells in the striatum and midbrain (eg, ventral tegmental area [VTA]). In addition to regulating the clearance of extracellular striatal DA, the DAT modulates the signal-to-noise ratio of DA neurotransmission and affects presynaptic DA levels.<sup>13</sup> Critically, DAT is regulated by extracellular DA levels. Specifically, DA synthesis depletion reduces striatal DAT density and function,<sup>14,15</sup> highlighting compensatory DAT downregulation to adjust to reduced DA concentrations. Similarly, DAT downregulation was seen in surviving midbrain DA neurons after the loss of striatal DA terminals.<sup>16</sup> Collectively, these findings highlight plastic changes in DAT levels depending on striatal DA availability.

Despite these preclinical data, evidence from human studies is inconclusive. In vivo evidence from positron emission tomography (PET) or single-photon emission computed tomography (SPECT) studies in MDD has been inconsistent. A recent meta-analysis revealed no significant associations.<sup>17</sup> However, 11 of the included 12 studies (91.7%) used SPECT, which has poorer spatial resolution compared with PET. Moreover, some of the tracers used (eg, [<sup>123</sup>I]β-CIT) have incomplete specificity for DAT vs the serotonin transporter, which complicates interpretations. This meta-analysis revealed considerable heterogeneity across studies, and it is unclear whether clinical heterogeneity contributed to inconsistencies. Similarly, to our knowledge, few human postmortem studies have investigated DAT in MDD<sup>18</sup> and none have assessed striatal DAT levels in MDD.

## Key Points

**Question** Are individuals with major depressive disorder who are not taking medication characterized by lower dopamine transporter levels within the brain reward system compared with psychiatrically healthy control participants?

**Findings** In this cross-sectional study that analyzed positron emission tomography in 25 individuals with major depressive disorder and 23 healthy controls and postmortem data in 15 individuals with major depressive disorder and 14 healthy controls, major depressive disorder was linked to lower dopamine transporter levels in the dorsal striatum. In the imaging data, this dysfunction was exacerbated by more episodes of depression.

**Meaning** Decreased dopamine transporter availability might represent a compensatory downregulation because of low dopaminergic signaling within mesolimbic pathways.

Our goal was to address these limitations by using a highly selective DAT tracer (<sup>11</sup>C)altropine) in individuals with MDD who were not taking medication and demographically matched healthy controls. Compared with other DAT tracers, altropine offers several advantages, including a rapid and specific kinetics in DA-rich striatal regions and high selectivity for DAT.<sup>19,20</sup> Moreover, clinical heterogeneity was addressed by only including individuals with MDD who were not taking medication (mostly nonsmokers) who were characterized using clinical scales that captured constructs conceptually associated with DA, including anhedonia and the perception of entrapment. The latter was selected because of a convergence of preclinical and clinical findings. Preclinically, depression-like responses<sup>21,22</sup> and DAT downregulation<sup>1,8</sup> have been observed in stressful circumstances in which escape is blocked. These data have been complemented by human findings that chronic stressors characterized by the perception of being trapped in inescapable situations are particularly depressogenic<sup>23,24</sup> and are prospectively associated with depression onset<sup>25</sup> and reoccurrence.<sup>26</sup> Finally, DAT expression was evaluated in postmortem tissues from donors with MDD for independent corroboration. We hypothesized that, compared with healthy controls, MDD would be characterized by lower striatal DAT density, reflecting a compensatory downregulation due to chronically reduced DA signaling.

## Methods

### PET Study

#### Participants

Participants included 23 healthy controls and 25 individuals with MDD who were not taking medication (Table). Because DAT expression declines with age,<sup>30</sup> the age range was restricted to 18 to 45 years. Participants provided written informed consent according to a protocol approved by the Partners Healthcare institutional review board. Eligibility was established using the structured clinical interview for the *DSM-IV*<sup>31</sup> (eMethods and eTable 1 in the *Supplement*). At screening, participants were administered several clinical scales, including the 17-item Hamilton Depression Inventory.<sup>28</sup>

**Table.** Sociodemographic and Clinical Data for 23 Healthy Controls and 25 Participants With MDD

Characteristic	Participants, Mean (SD)		Statistics	P Value
	Control (n = 23)	MDD (n = 25)		
Age, y <sup>a</sup>	26.49 (7.26)	26.52 (5.92)	t = -0.02	>.98
Sex ratio (male/female)	10/13	6/19	$\chi^2 = 2.05$	>.15
Luteal, No. (%) <sup>b</sup>	5 (41.7)	6 (31.6)	$\chi^2 = 0.33$	>.55
Follicular, No. (%)	7 (58.3)	13 (68.4)	NA	NA
Education, y	15.67 (1.93)	16.40 (2.42)	t = -1.14	>.25
White, No. (%)	14 (60.9)	18 (72.0)	$\chi^2 = 0.67$	>.40
Never married, No. (%)	17 (73.9)	23 (92.0)	$\chi^2 = 2.82$	>.09
Average caffeine consumption, mg/d	116.93 (95.86)	126.78 (93.62)	t = -0.36	>.72
Caffeine consumed 24 h before the PET session, mg/d	77.55 (120.84)	103.60 (101.73)	t = -0.80	>.42
Current smokers, No. (%) <sup>c</sup>	2 (9.5)	3 (12.5)	$\chi^2 = 1.00$	>.75
Income, \$, No. (%) <sup>d</sup>	NA	NA	$\chi^2 = 0.93$	>.25
<50 000	13 (56.5)	16 (69.6)		
50 000-100 000	8 (34.8)	6 (26.1)	NA	NA
>100 000	2 (8.7)	1 (4.3)		
Age of MDD onset, y	NA	16.46 (4.71)	NA	NA
Lifetime MDE <sup>e</sup>				
1 MDE	NA	3		
2-3 MDEs	NA	8	NA	NA
≥5 MDEs	NA	13		
HRSD	0.55 (1.01)	17.91 (3.79)	t = -20.79	<.001
BDI-II <sup>a</sup>	0.48 (1.31)	25.80 (8.65)	t = -8.90	<.001
SHAPS <sup>a</sup>	20.74 (5.54)	33.20 (4.11)	t = -13.89	<.001
EES <sup>a</sup>	1.44 (2.15)	19.64 (8.53)	t = -9.94	<.001

Abbreviations: BDI-II, Beck Depression Inventory-II<sup>27</sup>; EES, External Entrapment Scale<sup>22</sup>; HRSD, Hamilton Rating Scale for Depression<sup>28</sup>; MDD, major depressive disorder; MDE, major depressive episode; NA, not applicable; PET, positron emission tomography; SHAPS, Snaith Hamilton Pleasure Scale.<sup>29</sup>

<sup>a</sup> Variable assessed at the PET session.

<sup>b</sup> Missing for 1 healthy control.

<sup>c</sup> Missing for 2 healthy controls and 1 participant with MDD.

<sup>d</sup> Missing for 2 participants with MDD.

<sup>e</sup> Missing for 1 participant with MDD.

## Procedure

Approximately 10 mCi of [<sup>11</sup>C] altropine was injected intravenously (MDD: mean [SD], 9.00 [0.47]; controls: mean, [SD], 9.19 [0.34];  $P > .13$ ; range, 8.27-10.22 mCi) and serial PET images were acquired. After the scan, participants filled out various questionnaires, including the Beck Depression Inventory-II (BDI-II<sup>27</sup>) and the Snaith Hamilton Pleasure Scale (SHAPS<sup>29</sup>), to assess levels of depressive symptoms and anhedonia, respectively, as well as the External Entrapment Scale<sup>22</sup> (eMethods in the *Supplement*). Data regarding the apparatus and the data analyses are described in eMethods in the *Supplement*.

## Statistics

A multivariate analysis of covariance with hemisphere (left and right), striatal region (caudate, putamen, and NAc; eFigure 1 in the *Supplement*) and group (MDD and healthy controls) as factors and age as a covariate was run on binding potential (BPND). For the VTA, BPND values were averaged across the hemispheres, and entered in an analysis of covariance with group as a factor (covariate, age). Analyses were also repeated excluding age as a covariate and using partial volume corrected (PVC) data.<sup>32,33</sup>

For regions showing group differences in BPND, correlation analyses were performed with 3 clinical scales (BDI, SHAPS, and Entrapment Scale) and the number of lifetime

major depressive episodes (MDEs). For number of MDEs, because the distribution was skewed toward the right, we rebinned data by categorizing participants with MDD who reported 1 MDE, between 2 and 4 MDEs, and 5 or more MDEs.<sup>34</sup> For these analyses, healthy controls were included (MDE = 0), and Spearman rank correlations were performed. For the clinical scales, Pearson correlations were run in the MDD group only. All correlation analyses were conducted using age-residualized BPND values. Statistical significance was set at  $P < .05$ .

## Postmortem Study

Frozen tissue blocks containing the striatum from 15 individuals with depression and 14 psychiatrically healthy controls were obtained from the Douglas-Bell Canada Brain Bank (eMethods in the *Supplement*). All individuals with depression died by suicide and psychiatrically healthy controls died of natural or accidental causes. The cause of death for each participant was assessed by the Quebec Coroner's office.

## Results

### Imaging Studies

Groups were well matched with respect to sociodemographic variables, menstrual cycle, and caffeine consumption

(Table).<sup>27-29</sup> At screening, the MDD group had a mean (SD) 17-item Hamilton Rating Scale for Depression score of 17.91 (3.79; range: 12-28), indicating, on average, moderate depression; at the PET session, the MDD group reported a mean (SD) BDI-II score of 25.80 (8.65), also indicating moderate depression. Four participants with MDD (16.0%) had a current comorbidity of social phobia (secondary to MDD) and 1 participant with MDD (4.0%) had a current comorbidity of dysthymic disorders. Forty of 45 participants (88.9%) were nonsmokers (smoking status was missing for 2 healthy controls [8.7%] and 1 participant with MDD [4.0%]).

#### Group Differences in Striatal and Midbrain DAT BPND

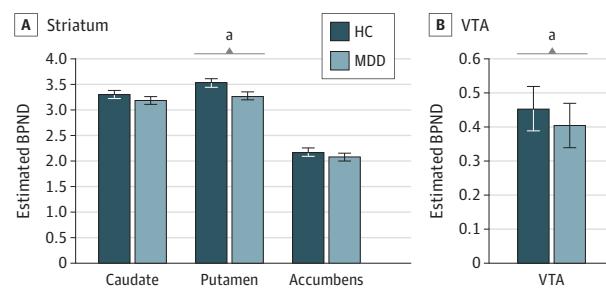
A group (healthy controls and MDD)  $\times$  region (caudate, putamen, and NAc)  $\times$  hemisphere multivariate analysis of covariance (covariate, age) yielded a significant main effect of region (Wilks  $\lambda$  [2,44] = 34.36;  $P < .001$ ) because of significantly higher BPND in the putamen than caudate, which in turn had significantly higher BPND than the NAc (all pairwise Bonferroni-corrected simple effects:  $P < .00003$ ). The effect of age (covariate) was also significant ( $F_{1,45} = 7.83$ ;  $P < .01$ ) because of decreasing striatal BPND with increasing age. Critically, the group  $\times$  region interaction (Wilks  $\lambda_{2,44} = 4.15$ ;  $P < .02$ ) was significant (Figure 1). Bonferroni-corrected simple effects revealed that, compared with controls, the MDD group had significantly lower BPND in the bilateral putamen ( $P < .03$ ; Cohen  $d = -0.66$ ), whereas groups did not differ in the caudate ( $d = -0.30$ ) and NAC ( $d = -0.23$ ) ( $P > 0.28$ ). When excluding age as a covariate, the group  $\times$  region interaction ( $F_{2,45} = 4.20$ ;  $P < .02$ ) and reduced bilateral putamen BPND in MDD ( $P < .03$ ; Cohen  $d = -0.62$ ) remained, indicating that age did not influence the group  $\times$  region interaction. Groups also differed in putamen BPND when using PVC data ( $t [46] = 2.04$ ;  $P < .047$ ;  $d = -0.59$ ).

For the VTA, analyses including ( $F_{1,48} = 6.04$ ;  $P < .02$ ;  $d = -0.71$ ) or excluding ( $t [46] = 2.45$ ;  $P < .02$ ;  $d = -0.71$ ) age as a covariate revealed a main effect of group due to overall lower BPND in MDD than controls (Figure 1B). However, this group difference was not seen when using PVC data ( $t [46] = 1.18$ ;  $P > .24$ ). For the putamen and VTA, all significant effects were confirmed when analyses were rerun only in nonsmokers (eResults in the Supplement).

#### Association With Number of Lifetime MDE

Among the participants with MDD, 3 (12.0%) reported 1 MDE, 8 (32.0%) had experienced between 2 and 4 MDEs, and 13 (52.0%) had experienced 5 or more MDEs. Spearman rank correlations among all participants (including healthy controls) indicated that, for the putamen ( $\rho = -0.36$ ;  $P < .01$ ; Figure 2) and the VTA ( $\rho = -0.40$ ;  $P < .01$ ), age-residualized BPND was negatively correlated with the numbers of lifetime MDEs. Because number of MDEs and age could be strongly related, hierarchical regressions entering age in the first step were performed (eResults in the Supplement). For both the putamen and VTA, number of MDEs continued to predict raw (non-age-residualized) BPND (putamen,  $\Delta R^2 = 0.111$ ;  $P < .02$ ; VTA,  $\Delta R^2 = 0.125$ ;  $P < .01$ ). The association of age with striatal

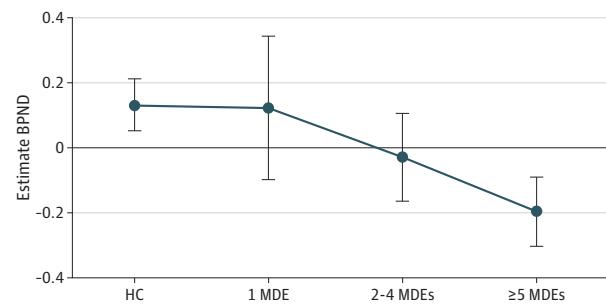
**Figure 1. Dopamine Transporter (DAT) Availability in Major Depressive Disorder (MDD)**



Age-residualized DAT availability (as assessed by binding potential [BPND]) in the healthy control (HC;  $n = 23$ ) and MDD ( $n = 25$ ) groups for striatal regions (A) and the ventral tegmental area (VTA) (B).

<sup>a</sup>  $P < .05$ .

**Figure 2. Dopamine Transporter (DAT) Availability as a Function of the Number of Major Depressive Episodes**



Age-residualized DAT availability (as assessed by binding potential [BPND]) in the mean (averaged across left and right) putamen as a function of the lifetime number of major depressive episodes (MDEs). HC indicates healthy control.

and midbrain DAT BPND is described in eResults in the Supplement.

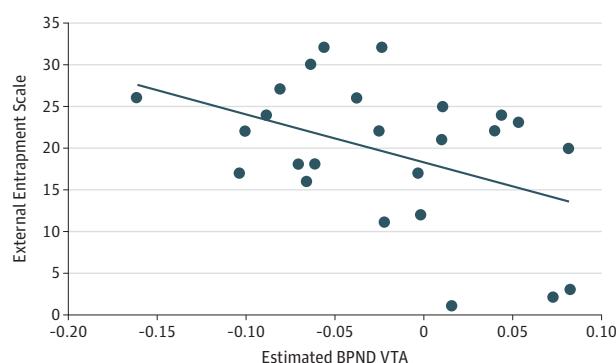
#### Association With Clinical Symptoms

Owing to the group findings reported above, correlations were performed between (1) mean age-residualized BPND in the putamen or VTA and (2) scores on 3 clinical scales (SHAPS, BDI, and Entrapment scale), leading to a total of 6 tests (Bonferroni correction:  $P < .05/6 = .0083$ ). Contrary to hypotheses, no correlations emerged for SHAPS scores ( $r_s < 0.38$ ;  $P > .06$ ). Mean age-residualized VTA BPND was negatively associated with scores on the External Entrapment Scale ( $r = -0.43$ ;  $P < .03$ ), indicating that an increasing perception of entrapment was associated with lower VTA BPND (Figure 3). Highlighting the specificity of this finding, External Entrapment Scale scores predicted VTA BPND even when entering BDI and SHAPS scores in the first step of a hierarchical regression ( $\Delta R^2 = 0.193$ ;  $\Delta F_{1,21} = 6.31$ ;  $P < .02$ ).

#### Human Postmortem Studies

In light of the PET findings, Western blots on putamen were used to measure DAT expression (the VTA was unavailable).

**Figure 3. Dopamine Transporter (DAT) Availability as a Function of Perceived Entrapment**



Scatterplot between external entrapment scale score and mean (averaged across left and right) ventral tegmental area (VTA) DAT availability within the major depressive disorder group. BPND indicates binding potential.

Owing to the hypothesized role of the NAc in MDD, Western blot analyses were also performed on the NAc (eResults in the Supplement). Depressed and control groups did not differ in age, sex ratio, postmortem time interval, or pH values (eTable 1 in the Supplement).

In the MDD group, tyrosine hydroxylase (TH) expression was significantly lower in the putamen ( $P = .007$ ;  $d = -1.11$ ) (Figure 4; eTable 2 in the Supplement). The expression of the mature form of DAT (80 kDa)<sup>35,36</sup> was significantly decreased in MDD ( $P = .005$ ;  $d = -1.20$ ). In addition, DAT 50 kDa ( $P = .02$ ;  $d = -0.99$ ) and 60 kDa ( $P = .01$ ;  $d = -0.99$ ), thought to represent intermediate glycosylated forms, were also significantly decreased. In contrast, the putative DAT precursor (40 kDa) did not differ. For TH and DAT, none of the covariates significantly affected group effects. Only the TH and 80 kDa group differences survived a Bonferroni correction ( $P < .05/5 = 0.01$ ).

## Discussion

Dopamine dysfunction has been implicated in MDD, but *in vivo* evidence has been elusive.<sup>1,17</sup> Abundant evidence indicates that DAT plays a pivotal role in synaptic DA regulation and reflects the integrity and function of the DA system.<sup>13</sup> Because of evidence that stress-associated animal models of depression and pharmacological manipulations depleting DA lead to reduced DAT levels, interpreted as reflecting a compensatory downregulation to regulate DA levels, we hypothesized that individuals with MDD who were not taking medication would show blunted striatal and midbrain DAT availability. We further hypothesized that such dysfunction would be greatest in individuals with MDD who reported elevated anhedonic symptoms and perceived entrapment (a construct associated with helplessness; eMethods in the Supplement). Several findings emerged.

First, compared with controls, the MDD group had significantly lower DAT availability in the bilateral putamen

and VTA. Notably, putamen and VTA availability were inversely associated with the lifetime number of MDEs. Although this latter observation is novel, prospective studies are needed to determine whether these findings represent a cumulative effect or potential premorbid marker of increased recurrence risk. Second, unlike controls, the MDD group failed to show age-associated declines in DAT availability. Third, contrary to our hypotheses, striatal and midbrain DAT availability was not moderated by anhedonic symptoms. However, in the VTA, a negative association between external entrapment scores and VTA DAT availability emerged (eResults in the Supplement). Thus, individuals with MDD who reported being trapped in putatively inescapable circumstances showed the lowest VTA DAT availability. These findings are intriguing, particularly considering prior reports that external entrapment scores prospectively predicted depression<sup>37</sup> and MDD reoccurrence<sup>26</sup> 12 to 16 months later. Fourth, a corroboration of reduced putamen DAT in MDD emerged in postmortem analyses.

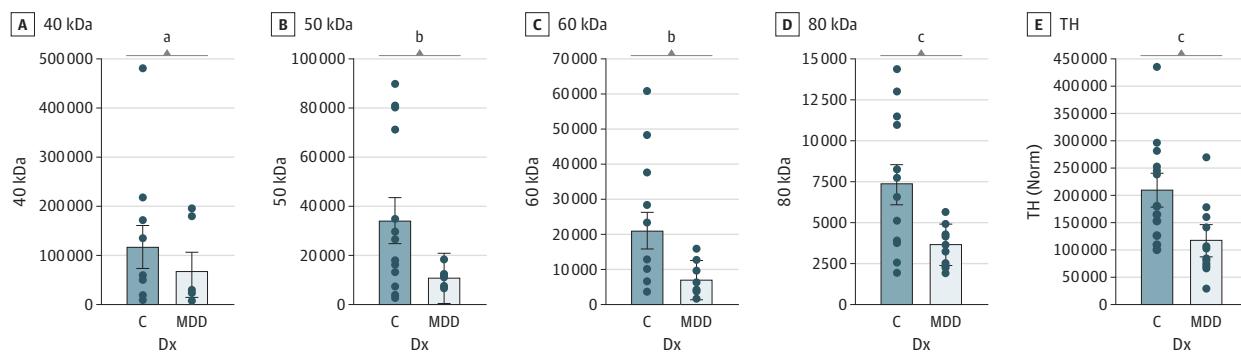
The current findings of lower striatal DAT availability in MDD agree with a prior PET study,<sup>38</sup> but findings have been inconsistent.<sup>17</sup> However, 11 of the 12 studies (91.7%) included in a prior meta-analysis,<sup>17</sup> as well as 14 of 15 studies (93.3%) included in a recent literature review,<sup>39</sup> used SPECT and suboptimal DAT tracers. Notably, the only PET study<sup>38</sup> reported reduced DAT availability in the putamen in a small MDD group ( $n = 9$ ). In addition to showing a 28-fold selectivity for DAT over the serotonin transporter (vs 1:1 for [ $^{123}\text{I}$ ]β-CIT and 3:1 for TRODAT<sup>19,20</sup>), altropine accumulates within 30 minutes almost exclusively to DA-rich striatal regions,<sup>40</sup> and the putamen:cerebellum ratio is 120:1, highlighting an exceptional degree of binding selectivity.<sup>40</sup> We speculate that using this highly selective PET tracer allowed us to more reliably probe DAT function in unmedicated MDD. Our findings fit prior reports of negative associations between dorsal striatal DAT availability and depressive symptoms in patients with MDD<sup>41</sup> and Parkinson disease.<sup>42</sup> Moreover, recovery from MDD has been associated with an increase in midbrain DAT availability,<sup>43</sup> and 6-week treatment with escitalopram increased striatal DAT availability by 20%.<sup>41</sup>

## Strengths and Limitations

The strengths of this study are that all individuals with MDD were unmedicated, had minimal degrees of comorbidity, and most were nonsmokers (21 of 24 [87.5%]). Focusing on an unmedicated sample is particularly important because of evidence that even brief treatment with selective serotonin reuptake inhibitors led to a 10% to 20% increase in striatal DAT availability.<sup>44</sup>

A novel finding is that, in MDD, the perception of being trapped in inescapable circumstances was associated with lower bilateral VTA availability. This link is intriguing, particularly in light of abundant evidence indicating that (1) exposure to stressful situations in which escape is blocked suppresses approach behavior and downregulates mesolimbic DA pathways<sup>1,8</sup> and (2) stressful life events characterized by entrapment are particularly depressogenic.<sup>29,32-34</sup> However, be-

Figure 4. Dopamine Transporter (DAT) and Tyrosine Hydroxylase (TH) Expression in Postmortem Analyses



Dopamine transporter and TH expression levels are decreased in the putamen of people with major depressive disorder (MDD). Box plots show the expression levels (mean and SE) for DAT (40, 50, 60, and 80 kDa) (A-D) and TH (60 kDa) (E). Protein levels are expressed as a normalized signal in fluorescence arbitrary units (LI-COR Odyssey CLx scanner). C indicates control group; Dx, diagnosis;

Norm, normalized signal.

<sup>a</sup> Not significant ( $P > .05$ ).

<sup>b</sup> Significant ( $P = .05$  to  $P = .01$ ).

<sup>c</sup> Highly significant ( $P = .01$  to  $P = .001$ ).

cause this correlation finding did not survive correction for multiple comparisons, replication is warranted.

Although PET imaging cannot pinpoint the source of DAT downregulation, increased inflammation and oxidative stress might be candidate mechanisms. Based on preclinical findings,<sup>16,45</sup> it is possible that increased inflammation and/or oxidative stress contributes to reducing DA signaling, which could eventually result in compensatory DAT downregulation. Consistent with this, mounting evidence points to increased inflammation in MDD,<sup>46</sup> and inflammation acutely decreases striatal DAT expression levels.<sup>47</sup> Moreover, and replicating prior findings,<sup>30</sup> DAT availability was negatively associated with age among the control, but not MDD, group. Notably, participants with MDD younger than the median (SD) age (21.72 [1.48] years) were indistinguishable from healthy controls (32.09 [6.70] years) and individuals with MDD (30.94 [4.86] years) older than the median age. Importantly, comparatively younger and older individuals with MDD did not differ in their number of lifetime MDEs (eFigure 2 in the Supplement) and age was not associated with depressive (Hamilton Rating Scale for Depression and BDI) or anhedonic (SHAPS) symptoms, indicating that these variables did not confound findings. Larger samples should evaluate whether the current findings reflect accelerated aging in MDD.<sup>48</sup> Because oxidative stress has been implicated in aging<sup>49</sup> and DA uptake has been found to be inhibited by oxidative stress,<sup>50,51</sup> it is possible that the current striatal and midbrain DAT downregulation might be due to increased oxidative stress in MDD. Two additional, and not reciprocally exclusive, potential mechanisms are suggested by the postmortem results. First, a disruption of DAT expression may be due to altered posttranslational modifications leading to a decrease of its active, mature form.<sup>35,36</sup> Our postmortem results show that levels of the immature DAT form were not altered in donors with MDD, whereas increasingly glycosylated forms up to the active 80 kDa form were decreased. These findings suggest that a disruption of glycosylation

mechanisms may lead to decreased DAT activity. Notably, the reduction of mature (glycosylated) forms of DAT has been described in surviving DA neurons after a loss of striatal DA terminals due to an oxidative injury in models of Parkinson disease.<sup>16</sup> Second, concurrent TH and DAT level decreases in MDD fit the possibility that DA terminals within the putamen may be reduced in MDD. If so, the result would be an overall decrease of DA tone. This possibility may be at odds with results showing normal levels of the immature forms of DAT, suggesting that dopaminergic terminals are still present in depression but express an immature, inactive form of DAT. However, it is plausible that both mechanisms may be involved and contribute to our findings. This possibility will be tested in future studies.

Despite strengths, including the inclusion of participants not taking medication, the use of a highly selective PET tracer, groups that were well matched for variables that could affect DA (eg, age, menstrual phase, smoking status, and caffeine use), and replication of the main finding in postmortem analyses, limitations should be emphasized. First, the MDD imaging sample was relatively young and moderately depressed; thus, it is unclear whether the current findings will replicate in different samples. This limitation is, at least partially, mitigated by parallel findings in postmortem studies, comprised of older donors with severe MDD that was likely to have led to suicide. Although results from postmortem studies cannot exclude that suicide, rather than MDD, is associated with DAT and TH level decreases, the convergence between imaging and postmortem results suggests otherwise. In addition, in the PET sample, suicidal ideation did not affect results (eResults in the Supplement). Second, information on pharmacological treatment for the participants included in postmortem studies was limited to toxicology data. It is possible that long-term treatment may affect DAT and TH expression. Again, the similarities between postmortem and imaging findings, the latter including participants not taking medication, mitigate this concern. Third, our

cross-sectional design prevented us from testing whether DAT downregulation is a potential cause or consequence of recurrence risk. Fourth, group differences in the VTA were not confirmed when using PVC data, indicating that caution should be used when interpreting VTA findings. Finally, it is unclear why BPND differences emerged in the dorsal but not ventral striatum, although it is important to emphasize that, in humans, DAT expression is highest in the dorsal striatum and weakest in the NAc.<sup>52</sup>

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#### Conclusions

These findings provide convergent evidence from in vivo molecular imaging and postmortem assays that MDD is characterized by DAT downregulation, likely reflecting a compensatory downregulation due to blunted DA signaling within reward-associated pathways, particularly with increasing numbers of prior MDEs.

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## Supplementary Online Content

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**eMethods.** Expanded Methodology

**eResults.** Expanded Results

**eDiscussion.** Expanded Discussion

**eFigure 1.** (A) Masks showing caudate (Cd, yellow), putamen (Pt, red), and nucleus accumbens (NAc, green) region-of-interest. (B) Masks showing the ventral tegmental area (VTA, blue) region-of-interest.

**eFigure 2.** (a) Scatterplot between age (at the day of the PET scan) and DAT availability (as assessed by binding potential (BPND)) in the bilateral putamen for the healthy control (gray triangles) and MDD (black dots) groups.

**eFigure 3.** Examples of Western blots for DAT and TH from control and MDD subjects.

**eTable 1.** Demographic and clinical information for the subject cohort included in the post-mortem study.

**eTable 2.** Results from human postmortem studies showing raw data, significance values and effect sizes (Cohen's d values) for DAT and TH.

This supplementary material has been provided by the authors to give readers additional information about their work.

## eMethods. Expanded Methodology

### PET Study

#### *Inclusion/Exclusion Criteria*

All participants were right-handed and reported no medical or neurological illnesses, no contraindications to MRI, no lifetime substance dependence and no substance abuse in the past year.

Additional exclusion criteria for the MDD group included use of any psychotropic medication in the past 2 weeks (6 weeks for fluoxetine, 6 months for dopaminergic drugs) and a psychiatric history of other major axis I disorders (except social and generalized anxiety if secondary to MDD). Exclusion criteria for controls included current or history of psychiatric illnesses, a family history of mood disorders or psychosis, and lifetime use of psychotropic medication.

Participants meeting study criteria were scheduled for separate fMRI<sup>1</sup> and PET sessions, which took place, on average 15.54 (SD: 11.43) days and 25.19 (SD: 22.69) days after the clinical screening session, respectively. The healthy control (30.26±24.86) and MDD (20.52±19.86) group did not differ in days between the SCID and PET sessions ( $t(46)=1.51, P>0.13$ ). Sample size was determined after considering effect sizes in prior SPECT/PET studies targeting DAT in MDD (e.g., <sup>2,3</sup>), acknowledging that inconsistencies have been report in this literature<sup>4</sup>.

#### *External Entrapment Scale*

External entrapment refers to the “perception of things in the outside world that induce escape motivation” (p. 589)<sup>5</sup>. The External Entrapment Scale was specifically administered only for the PET session due to *a priori* hypothesis that it might capture individual differences in DAT availability. This hypothesis was motivated by preclinical findings indicating that exposure to chronic inescapable stressors – such as prolonged immobilization stress<sup>6</sup>, chronic psychosocial stress<sup>7,8</sup>, and early maternal

separation<sup>9</sup> – resulted in decreased striatal DAT levels, which persisted for weeks after the stress termination.

The External Entrapment scale included 10 items, which were rated on a 5-point scale (0: never, 1: rarely, 2: sometimes, 3: mostly, 4: always). Examples of items are: *I am in a situation I feel trapped in; I am in a relationship I can't get out of; I can see no way out of my current situation; I feel trapped by my obligations*). Higher scores indicate more feelings of entrapment. The Cronbach's Alpha for the current sample was excellent ( $\alpha=0.95$ ; MDD group only:  $\alpha=0.88$ ). The construct of entrapment overlaps with the concept of helplessness. Consistent with this suggestion, various items of the external entrapment scales map onto helplessness (e.g., *I feel powerless to change things; I feel trapped by my obligations*) and robust positive correlations between external entrapment and helplessness scores (e.g.,  $r=0.52$ ) have been reported<sup>10</sup>. Moreover, it has been proposed that perceptions of entrapment (particularly after defeating experiences) trigger a psychobiological “helplessness script,” hypothesized to be evolutionarily drive to facilitate submissive behaviors<sup>11,12</sup>. In the current MDD sample, External Entrapment Scale scores did not correlate with either depression severity (BDI scores assessed at the PET session; Pearson  $r=0.23$ ,  $P>0.20$ ,  $N=25$ ) or anhedonia (SHAPS scores assessed at the PET session; Pearson  $r=0.08$ ,  $P>0.35$ ,  $N=25$ ), indicating that the External Entrapment Scale probed a non-overlapping construct.

#### *Procedure*

After re-screening for PET compatibility, participants were positioned in the gantry of the PET camera, and head alignment was made, relative to the canthomeatal line, using projected laser lines. A thermoplastic mask was fitted to the participant's face to reduce head movement. A peripheral venous catheter was inserted for radiopharmaceutical injection.

### *Apparatus*

An ECAT EXACT HR+ (CTI, Knoxville, TN) PET camera was used to assess [<sup>11</sup>C]altropane binding (3D mode, 63 contiguous 2.4 mm slices, 2.06 x 2.06 mm transaxial grid). To facilitate co-registration of PET data into stereotaxic space, structural MRI data were acquired on a 3T Siemens Tim Trio system (Siemens Medical Systems, Iselin, N.J.) equipped with 32-channels. High-resolution structural data were acquired using a T1-weighted magnetization-prepared rapid acquisition with gradient multi echo (MPRAGE) imaging sequence [time (TR) = 2200 ms; echo times (TE) = 1.54, 3.36, 5.18 and 7 ms; field of view = 230 mm; voxel dimensions = 1.2 x 1.2 x 1.2 mm<sup>3</sup>; 144 slices].

For the PET scan, (approximately) 10 mCi of [<sup>11</sup>C]altropane was injected intravenously over 20-30 sec. Images were acquired over 60 minutes in 39 frames of increasing duration (8 frames of 15 sec, 4 frames of 60 sec, 27 frames of 120 sec). PET images were reconstructed using a filtered back-projection algorithm with physical corrections applied for photon scatter and attenuation, random coincidences, system deadtime, and detector inhomogeneity. Next, motion-corrected frames were summed and FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) was used to determine the rigid body transformation matrix to align the PET and subject-specific MPRAGE. FSL non-linear registration was used to determine the individual warping (and its inverse) of each MPRAGE to a common reference space (Montreal Neurological Institute, MNI) where volume-of-interest masks were delineated. The derived transformations were then concatenated and applied to the volume-of-interest masks for extraction of regional time-activity curves from the dynamic PET images in native subject space.

### *Data reduction/analyses*

For PET analyses, the multilinear reference tissue model<sup>13</sup> was used to calculate regional BPND<sup>14</sup>, using the cerebellum (excluding the vermis) as reference region<sup>15,16</sup>. BPND was estimated for left and right dorsal (caudate nucleus, putamen) and ventral (NAc) striatum, which were defined as volumes of

interest using a 50% probabilistic threshold applied to the Harvard-Oxford Subcortical Probabilistic Atlas available in FSL<sup>17,18</sup>. The mask for the ventral tegmental area (VTA) was defined as a data-driven functional volume of interest based on the a priori hypothesis of group differences in [<sup>11</sup>C]altropane BPND in this brain region. The overall mask was manually traced using well-established guidelines<sup>19,20</sup>. This mask was mirrored and bisected to generate lateralized left and right volumes of interest which were visually confirmed to overlay the VTA on the MNI atlas. The left and right masks were symmetric, and each consisted of 81 voxels, each with 2 mm isotropic dimensions. The VTA masks are depicted in **eFigure 1**.

### Postmortem Study

#### *Human Subjects*

Frozen tissue blocks containing the putamen (as well as the nucleus accumbens) from depressed individuals who died by suicide (n=15) and psychiatrically healthy controls (n=15) who died by natural or accidental causes were obtained from the Douglas-Bell Canada Brain Bank. Brain samples were dissected at 4 °C, snap-frozen in liquid nitrogen and stored at -80 °C following standard procedures. Cause of death for each subject was assessed by the Quebec Coroner's office. After brain collection, information on the subjects' mental health was obtained using psychological autopsies using the Structured Clinical Interviews for DSM-IV axis I<sup>21</sup> (see below for detail). Brain tissue samples from all subjects were assessed for the absence of pathological processes by a neuropathologist. Written informed consent was obtained from next of kin for all subjects, and the Douglas Institute Research Ethics Board approved this study.

#### *Psychological Autopsy*

For the MDD group, individuals who met DSM-IV diagnostic criteria for MDD in the 6 months before their death and who committed suicide were included. For controls, individuals who died by natural cause or accidents without evidence of lifetime psychopathology and matching the MDD group with respect to demographic variables (e.g., gender, age, ethnicity) and variables that could affect post-mortem analyses (e.g., post-mortem intervals (in hours), pH) were included. As summarized in prior publications from the McGill Group for Suicide Studies (e.g., <sup>21-24</sup>), psychiatric diagnoses were made using the psychological autopsy method, which has been extensively validated (e.g., <sup>25,26</sup>). This approach involves interviews with one or more family members who were best acquainted with the deceased. Psychiatric diagnoses were established using the SCID adapted for proxy-based interviews. Diagnostic information was supplemented by coroner's notes and medical records, yielding a written case history for each deceased. Such case histories were then reviewed by a clinical panel, which was tasked to reach a consensus with respect to DSM-IV diagnoses. In prior studies from the McGill Group for Suicide Studies (e.g., <sup>22</sup>), kappa coefficients between two or more clinical raters for key diagnoses were excellent (major depression: 0.96; alcohol abuse/dependence: 0.98; drug abuse/dependence: 1.00; bipolar disorder: 1.00).

#### *Protein extraction and Western Blotting*

The putamen (as well as the nucleus accumbens) was dissected from tissue blocks and sections were cut using a cryostat. Tissue sections were homogenized using a mortar and pestle with added RIPA buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 1% Triton-X 100, 0.5% Na deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 10mM ethylenediaminetetraacetic acid (EDTA)) plus protease inhibitor. Samples were then transferred to eppendorf tubes, sonicated 3x at 50% power, and then centrifuged in for 20 min at 16,000 x g to remove cell debris. The supernatant was aspirated and used for western blots. The protein concentration was determined by DC assay (Biorad) in a 96 well plate. Western blots for both

DAT and tyrosine hydroxylase (TH) were run on 4-12% bis-tris gradient gels (NuPage, ThermoFisher) in MOPS buffer 200V (constant voltage) for 1 hour. 20 $\mu$ g of protein/sample was loaded after denaturing with 10mM DTT at 75C for 10 min in loading buffer. The charge separated proteins were transferred from the gel to a PVDF membrane at 30V (constant voltage) for 1.5 hours. After transfer, the blots were incubated with blocking buffer (Odyssey Blocking Buffer) for 1hr and then incubated overnight with 1:1000 anti-DAT antibody (rabbit, EMD Millipore AB5802) or 1:2000 anti-TH antibody (sheep, Pel-Freeze P60101) overnight on a shaker at 4°C. The blots were washed 4 times in sodium phosphate buffer + 0.2% TWEEN (PBST) and then incubated with 1:20,000 secondary antibody (LI-COR) respective to the primary antibody used for 2 hours at room temperature on a shaker and protected from light. The blots were washed 4 times in PBST before drying and imaging.

#### *Primary Antibodies*

The primary antibodies used were anti-DAT antibody (rabbit, EMD Millipore AB5802) or 1:2000 anti-TH antibody (sheep, Pel-Freeze P60101). Both antibodies were tested for specificity using 30 um thick rat sections. In addition, both antibodies were titrated using 40 ug of sample at the following concentrations (1:500, 1:1000, 1:2000). Total protein loads of (10, 20 and 40 ug) were tested for each antibody. The distribution of DAT was homogenously distributed through the putamen. This was tested using post-fixed slide mounted sections from our cohort.

#### *Western Blot Analysis*

Western blots were imaged after drying on a LI-COR Odyssey CLx scanner. Images were analyzed using Image Studio (LI-COR) using local background subtraction. DAT or TH signals were normalized to the highest median valosin-containing protein (VCP) signal, labeled with a different fluorophore.

### *Statistical analysis*

Differences between groups relative to the main outcome measures in the putamen (and nucleus accumbens) were assessed for statistical significance using an ANCOVA stepwise linear regression process. Effect sizes were calculated using Cohen's d values. Statistical analyses were performed using JMP v5.0.1a (SAS Institute Inc., Cary, NC). Age, gender, postmortem time interval, substance dependence, and exposure to benzodiazepines were tested systematically for their effects on the main outcome measures and included in the model if they significantly improved the model goodness of-fit. Note that cause of death for the MDD group was suicide for all subjects.

### **eResults. Expanded Results**

#### PET Study

##### *Group analyses restricted in non-smokers*

For both the striatal and VTA ROIs, all significant effects reported in the main text were confirmed when analyses were re-run only in non-smoking healthy control (N=19) and MDD (N=21) participants. For striatal ROIs, the *Group* x Region interaction was confirmed (Wilks' Lambda (2,36)=3.85,  $P<0.031$ ); Bonferroni-corrected simple effects confirmed that, relative to non-smoking controls, non-smoking MDD individuals had significantly lower BPND in the bilateral putamen ( $P<0.006$ ; Cohen's d=-0.95) and trending lower BPND in the bilateral caudate ( $P=0.076$ ; d=-0.61), whereas groups did not differ in the NAc ( $P>0.21$ ; d=-0.44). Similarly, for the VTA, BPND was lower in non-smoking MDD than controls ( $t(38)=2.31$ ,  $P<0.027$ , d=-0.73). When using partial volume corrected data, the group difference in putamen BPND was confirmed ( $t(28)=2.87$ ,  $P<0.007$ , d=-0.91), whereas for the VTA it was not ( $P>0.25$ ).

##### *Effects of age on striatal and midbrain DAT BPND*

Prior studies have shown that DAT levels decline with age<sup>27</sup>, but no study has evaluated this effect in MDD. Linear regressions were performed to determine the effects of age on DAT binding. In controls, DAT BPND in all striatal regions was negatively correlated with age (left caudate:  $r=-0.60$ ,  $P<0.003$ ; right caudate:  $r=-0.57$ ,  $P<0.004$ ; left putamen:  $r=-0.46$ ,  $P<0.029$ ; right putamen:  $r=-0.49$ ,  $P<0.019$ ; Left NAc:  $r=-0.49$ ,  $P<0.018$ ; right NAc:  $r=-0.51$ ,  $P<0.014$ ). Conversely, no age effects were seen in MDD (all  $P>0.18$ ; **eFigure 2a**), although correlations for the two groups were not significantly different (Fisher's test: all  $Z<1.64$ , all  $P>0.05$ ). For the VTA, correlations for neither group were significant (all  $P>0.15$ ).

In an alternative approach, participants were divided using a median split approach (median age across groups: 24 years old). Relative to healthy controls below the median age, MDD individuals below the median age showed significantly lower DAT BPND in the putamen ( $t(22)=2.40$ ,  $P<0.025$ ;  $d=-0.98$ ; **eFigure 2b**), VTA ( $t(22)=2.89$ ,  $P<0.009$ ;  $d=-1.18$ ), and caudate (trend:  $t(22)=1.74$ ,  $P=0.096$ ;  $d=-0.71$ ); groups did not differ when considering participants over the age of 24 (all  $P>0.70$ ). Moreover, for healthy controls—but not the MDD group ( $P>0.57$ )—participants older than 24 had significantly lower BPND relative to participants younger than 24 in the caudate [ $t(21)=2.62$ ,  $P<0.016$ ;  $d=-1.08$ ], VTA [ $t(21)=2.26$ ,  $P<0.035$ ;  $d=-0.97$ ], and putamen [trend:  $t(21)=1.81$ ,  $P=0.085$ ;  $d=-0.75$ ]. Of note, MDD individuals under the age of 24 (mean age $\pm$ SD:  $21.72\pm1.48$ ) did not differ from healthy controls over the age of 24 ( $32.09\pm6.70$ ) in any region (all  $P>0.15$ ).

#### *Relations between number of lifetime major depressive episode and DAT BPND*

In the current PET sample, age and number of episodes were moderately correlated (Pearson  $r: 0.474$ ,  $P<0.035$ ), suggesting that these two variables shared only 22.4% of their variance. Of note, the variable used to code number of episodes (0: healthy controls, 1: one lifetime MDE, 2: between 2 and 4 lifetime MDE; 3: more than 5 lifetime MDE) was not correlated with age ( $r = 0.090$ ,  $P>0.54$ ). In line with these observations, hierarchical regression analyses entering age in the first step and coding for lifetime

MDE (from 0 to 3) in the second step confirmed that number of MDEs continued to predict raw (non-age-residualized) BPND (putamen:  $\Delta R^2=0.111$ ,  $P<0.016$ ; VTA:  $\Delta R^2=0.125$ ,  $P<0.014$ ).

#### *Effects of suicidal ideation on striatal and midbrain DAT BPND*

One of the most important findings emerging from this study is the convergence between the PET and post-mortem analyses, both pointing to lower DAT density in MDD relative to healthy controls. It is important to emphasize, however, that for the post-mortem analyses, all MDD donors died by suicide, raising the possibility that suicide, rather than MDD, may be associated with DAT and TH decreases. As a preliminary test of this hypothesis, we compared DAT BPND in striatal regions and the VTA between MDD subjects reporting no suicidal ideation (i.e., scored a “0” on item 9 of the Beck Depression Inventory (“*I don't have any thoughts of killing myself*”; n=10) and those reporting some suicidal ideation (i.e., scored a “1” on item 9 of the Beck Depression Inventory (“*I have thoughts of harming myself, but I would not carry them out*”; n = 11). Note that four MDD participants had missing BDI, and that no participants scored higher than “1” on the BDI item 9, highlighting limited severity range. For striatal regions, a *MDD subgroup* (no vs. some suicidal ideation) x *Region* (caudate, putamen, NAc) x *Hemisphere* MANCOVA (covariate: Age) yielded no significant findings involving *MDD subgroup* (all Wilks' Lambda (2,44)<2.11,  $P>0.15$ ). A similar *MDD Subgroup* x *Hemisphere* MANCOVA revealed no effects involving *MDD subgroup* for the VTA (all Wilks' Lambda (1,18)<2.85,  $P>0.11$ ).

#### Human Postmortem Study

##### *Healthy controls*

In healthy controls, TH western blots showed a single band at 60 kDa (see **Figures 4** in the main text). DAT showed several bands corresponding to 40, 50, 60/65 and 80 kDa (**eFigure 3**). These bands have been shown to correspond to the DAT non-glycosylated precursor (40 kDa) and to (increasingly)

glycosylated forms of DAT (50, 60/65 and 80kDa). The 80 kDa DAT is recognized as the mature form of the molecule<sup>28,29</sup>.

#### *Post-mortem analyses of nucleus accumbens DAT*

In the *in vivo* PET analyses, groups did not differ in BPND in the nucleus accumbens. To further evaluate the role of the nucleus accumbens in MDD, we performed analyses probing DAT expression in this region. Relative to healthy controls, the MDD group had significantly lower expression of the immature (non-glycosylated) form of DAT (40 kDa) ( $F(1,25)=4.69$ ,  $P<0.04$ ; Cohen's d value: -0.83). Alcohol dependence/substance at death ( $F(1,25)= 4.95$ ,  $P<0.036$ ) was also associated with expression of the immature (non-glycosylated) form of DAT (40 kDa), with subjects with alcohol exposure having higher 40kDa expression than those without alcohol exposure. Critically, differences between the MDD and healthy control groups became statistically more significant when accounting for alcohol exposure ( $F(1,24)=11.63$ ,  $P<0.0023$ ). Unlike findings in the putamen, no group differences emerged for the mature (glycosylated) form of DAT (80 kDA, 55-60 kDA or 48 kDA) (all  $P>0.34$ ).

#### **eDiscussion.** Expanded Discussion

Unlike findings in the putamen, where the PET and post-mortem analyses replicated each other, for the nucleus accumbens, group differences emerged only in the post-mortem analyses. Specifically, relative to demographically matched healthy controls, individuals with MDD who died by suicide were characterized by significantly lower expression of the immature (non-glycosylated) form of the DAT (40kDA). In light of prior evidence that the immature form is associated with less efficient DAT<sup>30,31</sup>, it is possible that the difference emerging from the post-mortem analyses were too subtle to be detected in the PET analyses.

#### Lower VTA DAT BPND in MDD

In addition to lower BPND in the putamen, the MDD group was characterized by lower BPND in the VTA compared to healthy controls. In situ hybridization and other techniques have demonstrated robust expression of DAT in the VTA in rats, non-human primates and humans (e.g., <sup>32-34</sup>). Relevant to the current PET findings, rat strains bred for increased vulnerability to depression showed reduced DAT expression in the VTA, among other regions<sup>33</sup>. In a similar vein, adverse rearing environments (maternal deprivation), which has been found to reduce DA signaling within midbrain and striatal regions (for review, see <sup>35</sup>) induced anhedonic behavior and downregulation of both DAT mRNA and protein in rats<sup>36</sup>. In rats bred for increased vulnerability to depression, VTA DAT reduction was interpreted as reflecting “an adaptive response to abnormally low levels of DA in the mesolimbic pathway”<sup>33</sup> (p. 917), which is consistent with our interpretations.

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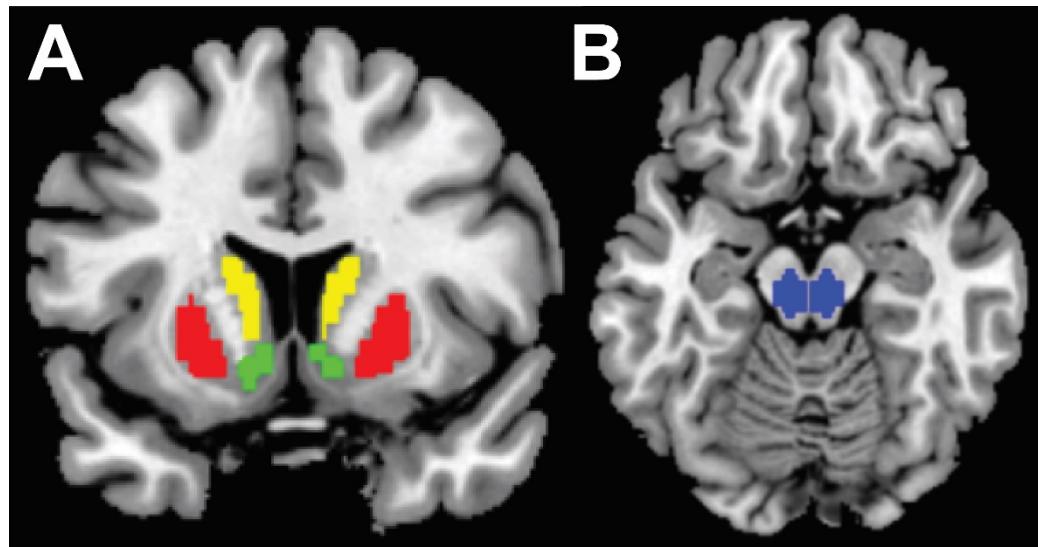
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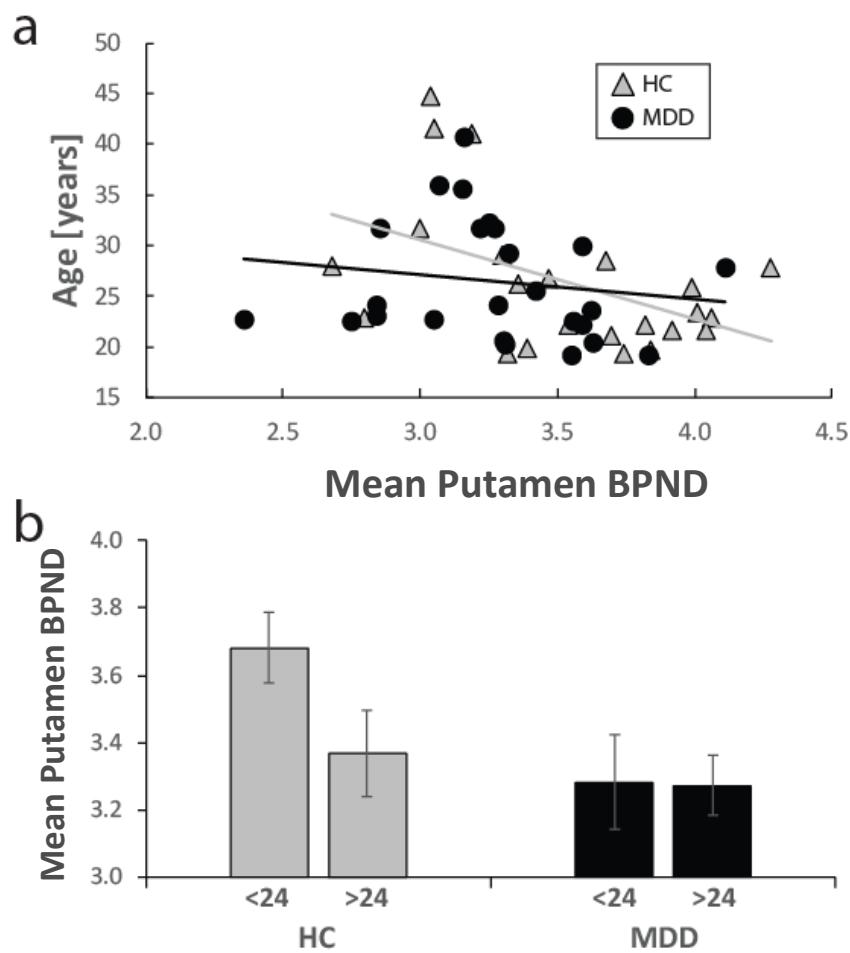
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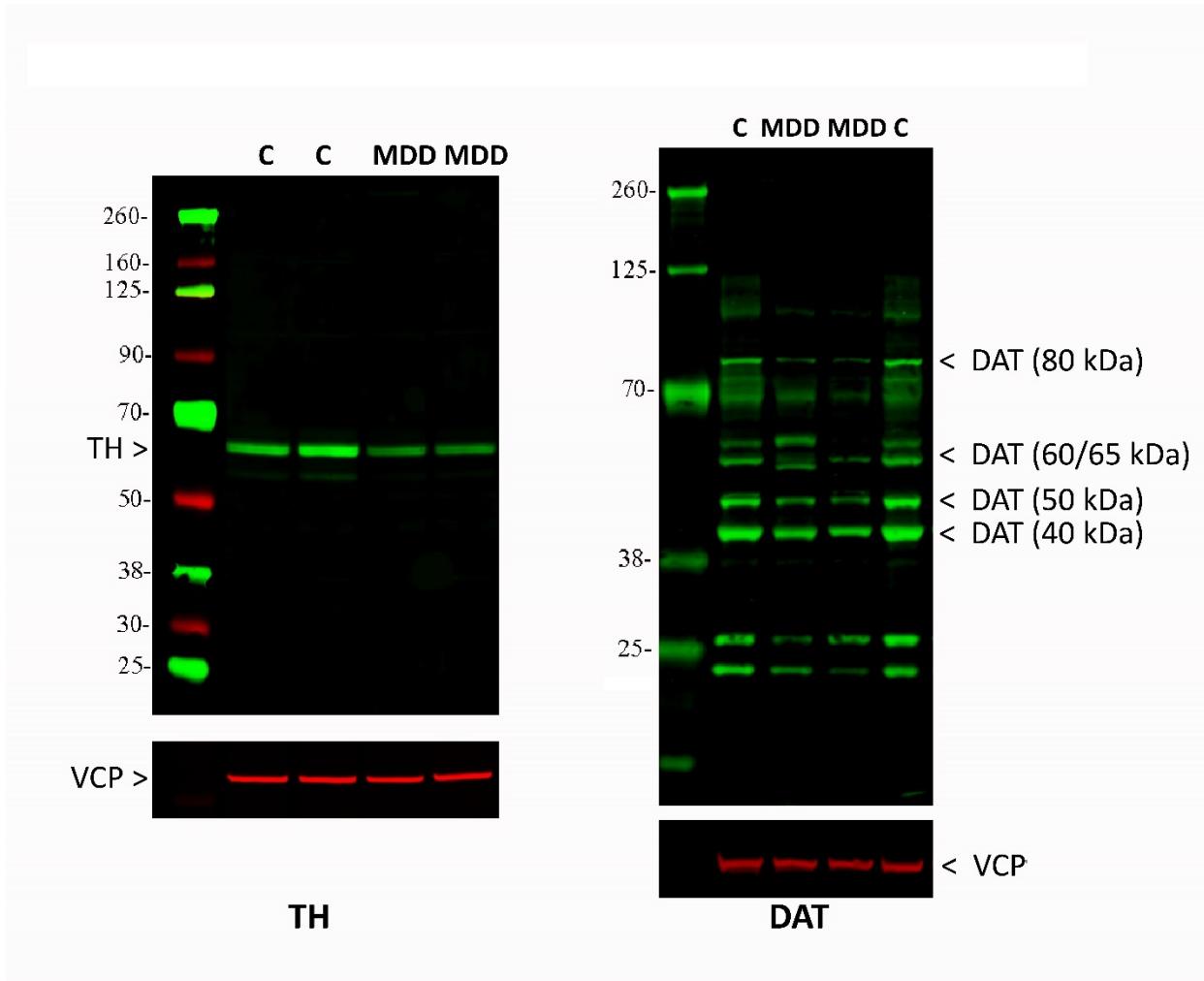
**eFigure 1:** **(A)** Masks showing caudate (Cd, yellow), putamen (Pt, red), and nucleus accumbens (NAc, green) region-of-interest. **(B)** Masks showing the ventral tegmental area (VTA, blue) region-of-interest.



**eFigure 2: (a)** Scatterplot between age (at the day of the PET scan) and DAT availability (as assessed by binding potential (BPND)) in the bilateral putamen for the healthy control (gray triangles) and MDD (black dots) groups. For HC ( $r = -0.47, P < 0.025$ ), but not MDD ( $r = -0.16, P > 0.45$ ) subjects, bilateral putamen BP was inversely related to age; **(b)** Bilateral putamen DAT availability for healthy controls (HCC) and individuals with major depressive disorder (MDD), split into subgroups younger or older than the median age (24 years old) for the entire sample. Among MDD individual below the median age ( $n=12$ ), 3 (25%) were experiencing their first MDE, 3 (25%) reported between 2 and 4 MDEs, and 6 (50%) reported 5 or more lifetime MDEs. Among MDD individuals above the median age, 5 (38.5%) reported between 2 and 4 MDEs, and 8 (61.5%) reported 5 or more lifetime MDEs ( $\chi^2 = 3.72, df = 2, P > 0.15$ ).



**eFigure 3:** Examples of Western blots for DAT and TH from control and MDD subjects. Note distinct DAT bands at 40, 50, 60/65 and 80 kDa. Decreases MDD were detected for TH and for 50, 60/65 and 80 kDa bands for DAT.



**eTable 1:** Demographic and clinical information for the subject cohort included in the post-mortem study. Post-mortem time interval (PMI) expressed in hours. HC, unaffected control subjects; MDD, subjects with major depression disorder.

Axis I	Brain ID	Age	PMI (h)	pH Value	Gender	Race	Cause of death	Substance dependence
HC	A	42	20	6.62	Male	Caucasian	Natural	-
HC	C	47	12	6.49	Male	Caucasian	Natural	-
HC	D	28	27	6.32	Male	Caucasian	Accident	-
HC	E	41	24	6.00	Male	Caucasian	Natural	-
HC	F	31	29.5	6.67	Male	Caucasian	Accident	-
HC	H	46	19.5	6.42	Male	Caucasian	Natural	-
HC	J	27	20.5	6.55	Male	Caucasian	Natural	-
HC	M	33	26.5	6.82	Male	Caucasian	Natural	-
HC	T	15	27	6.72	Male	Caucasian	Accident	-
HC	V	63	13	6.84	Male	Caucasian	Accident	-
HC	Z	72	17	6.10	Female	Caucasian	Natural	-
HC	AA	43	27	6.70	Male	Caucasian	Natural	-
HC	BB	55	21	6.70	Male	Caucasian	Natural	-
HC	CC	26	12	6.75	Male	Caucasian	Accident	-
MDD	B	26	21.5	5.50	Male	Caucasian	Suicide	Yes
MDD	G	42	21	6.40	Male	Caucasian	Suicide	-
MDD	I	45	20.5	6.57	Male	Caucasian	Suicide	Yes
MDD	K	39	25.5	6.60	Male	Caucasian	Suicide	Yes
MDD	L	22	11.5	6.35	Male	Caucasian	Suicide	Yes
MDD	N	18	27	6.22	Male	Caucasian	Suicide	-
MDD	O	39	19	6.00	Male	Caucasian	Suicide	-
MDD	P	48	21.5	6.79	Male	Caucasian	Suicide	Yes
MDD	Q	48	15	6.78	Male	Caucasian	Suicide	Yes
MDD	R	22	24	6.68	Male	Caucasian	Suicide	-
MDD	S	46	15	6.53	Female	Caucasian	Suicide	-
MDD	U	40	20	6.33	Male	Caucasian	Suicide	Yes
MDD	W	39	18.5	6.37	Male	Caucasian	Suicide	-
MDD	X	54	28.5	6.77	Female	Caucasian	Suicide	-
MDD	Y	55	26.3	6.50	Female	Caucasian	Suicide	-
HC	Mea	40.64	21.14	6.55	13 males		5 accidents	0
HC	SD	15.53	5.98	0.26	1 female		9 natural	
MDD	Mea	38.87	20.98	6.43	12 males		15 suicides	7
MDD	SD	11.73	4.81	0.34	3 females			
MDD	t-	0.35	0.08	1.10				
MDD	p-	.730	.937	.280	.598 <sup>a</sup>			

<sup>a</sup>Fisher's Exact test

**eTable 2:** Results from human postmortem studies showing raw data, significance values and effect sizes (Cohen's d values) for DAT and TH. Western blots were imaged Image Studio (LI-COR) using local background subtraction. DAT or TH signals were normalized to the highest median valosin-containing protein (VCP) signal, labeled with a different fluorophore. \* Significant using a Bonferroni correction involving 5 tests ( $p = 0.05/5 = 0.010$ ).

Protein levels in the putamen measured by fluorescent western blot								
		Control		MDD				
		Mean	SE	Mean	SE	t ratio	p value	Effect size
DAT	80 kDa	7363	798	3677	862	3.14	.0045*	1.15
	60 kDa	21065	3558	6944	3842	2.70	.0126	0.99
	50 kDa	34141	6377	10542	6888	2.51	.019	0.92
	40 kDa	116848	29294	61862	31642	1.28	.2145	0.47
TH	60 kDa	192436	18528	118476	17348	2.92	.0071*	1.06