Supplementary Online Content

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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[1] 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., [2,3]), acknowledging that inconsistencies have been report in this literature[4].

External Entrapment Scale

External entrapment refers to the "perception of things in the outside world that induce escape motivation" (p. 589)[5]. 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[6], chronic psychosocial stress[7,8], and early maternal

separation[9] – 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 (α=0.95; MDD group only: α=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[10]. 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[11,12]. 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 [^{11}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^3; 144 slices].

For the PET scan, (approximately) 10 mCi of [^{11}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[13] was used to calculate regional BPND[14], using the cerebellum (excluding the vermis) as reference region[15,16]. 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[17,18]. 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 [^{11}C]altropane BPND in this brain region. The overall mask was manually traced using well-established guidelines[19,20]. 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[21] (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., [21–24]), psychiatric diagnoses were made using the psychological autopsy method, which has been extensively validated (e.g., [25,26]). 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., [22]), 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 homogeneized 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 ethylenediaminetriacetic 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. 20μg 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[27], 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 Ps>0.18; **eFigure 2a**), although correlations for the two groups were not significantly different (Fisher's test: all Z<1.64, all Ps>0.05). For the VTA, correlations for neither group were significant (all Ps>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 Ps>0.70). Moreover, for healthy controls–but not the MDD group (Ps>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±SD: 21.72±1.48) did not differ from healthy controls over the age of 24 (32.09±6.70) in any region (all Ps>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: ΔR^2=0.111, *P*<0.016; VTA: ΔR^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[28,29].

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 Ps>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[30,31], 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., [32–34]). Relevant to the current PET findings, rat strains bred for increased vulnerability to depression showed reduced DAT expression in the VTA, among other regions[33]. 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 [35]) induced anhedonic behavior and downregulation of both DAT mRNA and protein in rats[36]. 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"[33] (p. 917), which is consistent with our interpretations.

eReferences

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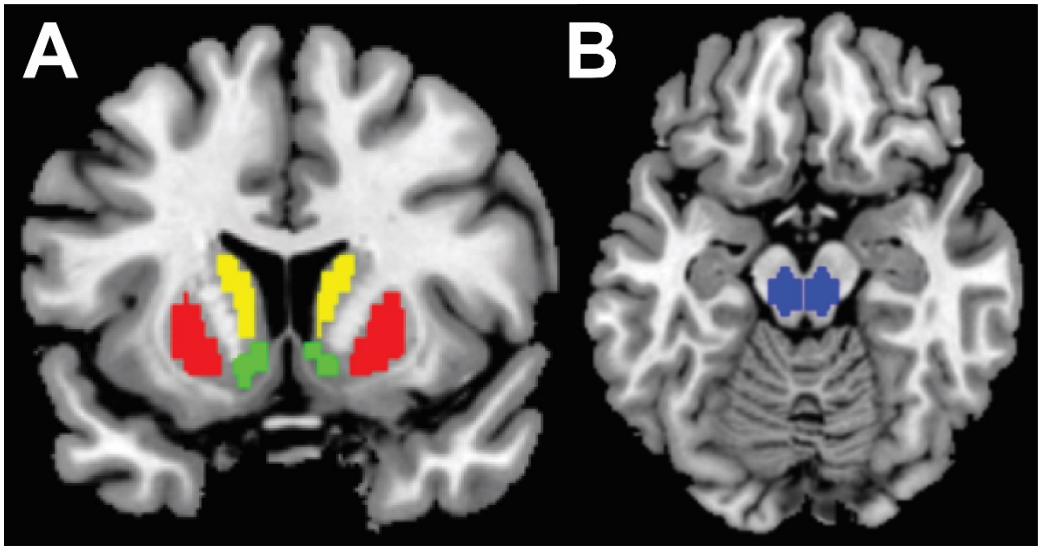
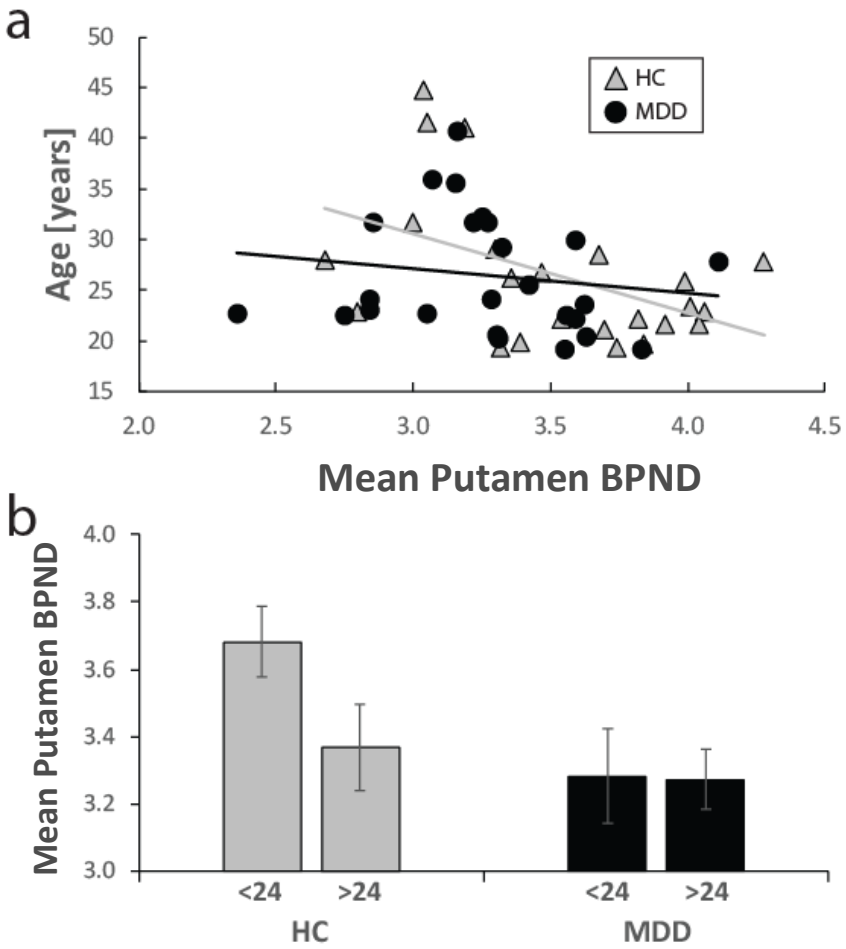
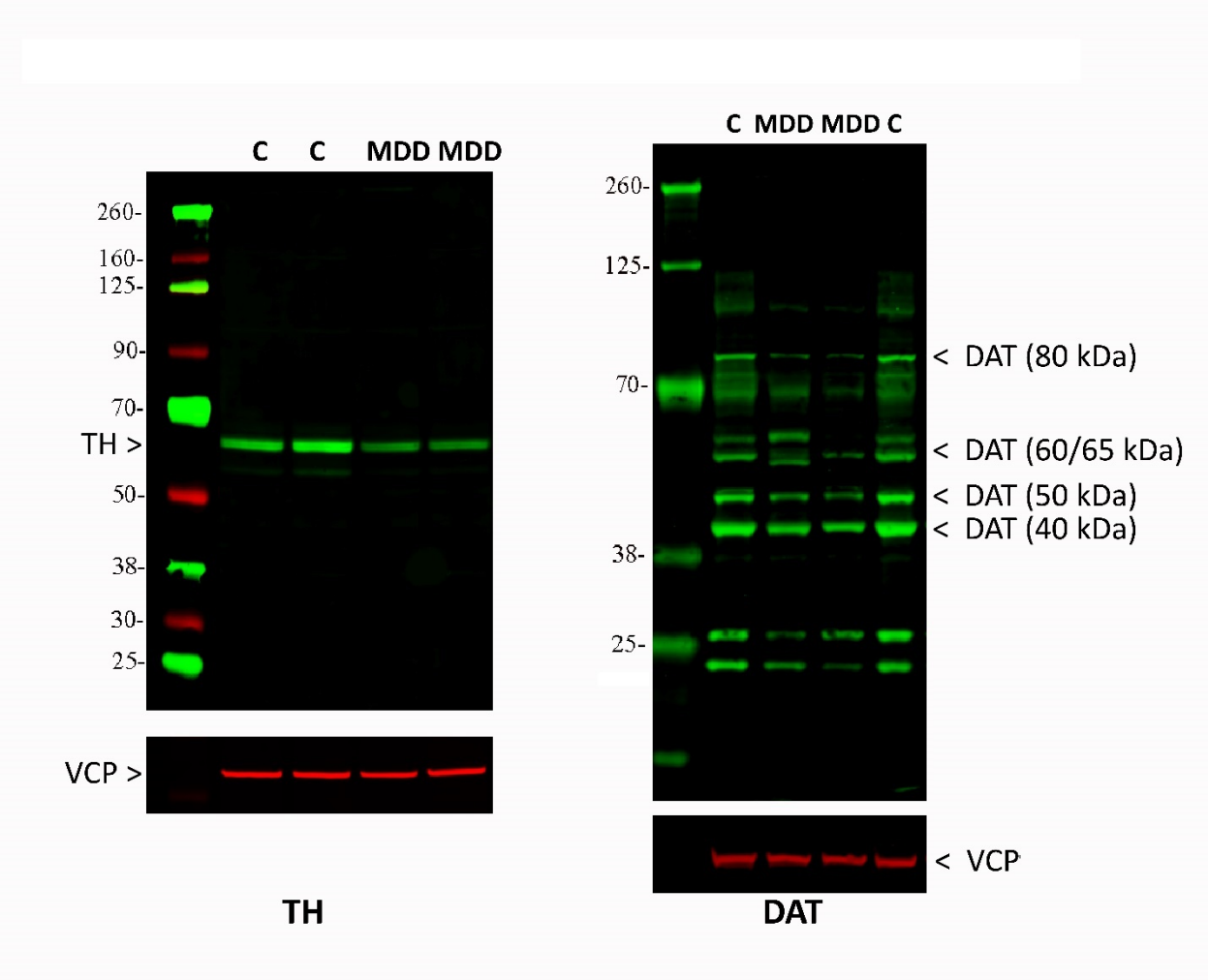
eFigure 1: (A) Masks showing caudate (Cd, yellow), putamen (Pt, red), and nucleus accumbens (NAc, green) region-of-interest. **(B)** Masks showing the ventral tegmental area (VTA, blue) region-of-interest.



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
	SD	15.53	5.98	0.26	1 female		9 natural	
MDD	**Mea**	38.87	20.98	6.43	12 males		15 suicides	7
	SD	11.73	4.81	0.34	3 females			
	t-	0.35	0.08	1.10				
	p-	.730	.937	.280	.598[a]			

[a]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