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Dysregulation of Striatal Dopamine Receptor Binding in Suicide

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Inconsistent evidence implicates disruptions of striatal dopaminergic indices in suicide and major depression. To determine whether there are alterations in the striatal dopamine system in suicide, we conducted a quantitative autoradiographic survey of dopamine transporter (DAT; [^{3}H]mazindol), D1 receptor ([^{3}H]SCH23390), and D2 receptor ([^{3}H]sulpiride) binding in the dorsal striatum *postmortem* from matched suicides and controls. Axis I and axis II psychiatric diagnosis, recent treatment history, and early life adversity (ELA) were determined by psychological autopsy. Mean DAT, D2, and D1 receptor binding did not differ in suicide. However, there was a positive correlation between D1 and D2 receptor binding in the dorsal striatum of control subjects ($R^2 = 0.31$, $p < 0.05$) that was not present in suicides ($R^2 = 0.00$, $p = 0.97$). In suicides and controls with reported ELA, there was no correlation between striatal DAT and D1 receptor binding ($R^2 = 0.07$, $p = 0.33$), although DAT and D1 receptor binding was positively correlated in subjects with no report of ELA ($R^2 = 0.32$, $p < 0.05$). After controlling for age, there were no significant ELA-related mean differences. Binding of D1 receptors and DAT throughout the striatum correlated negatively with age (D1 receptor: $R^2 = 0.12$, $p < 0.05$; DAT: $R^2 = 0.36$, $p < 0.001$). There appears to be an imbalance in dopaminergic receptor and transporter expression related to suicide that differs from that associated with ELA or age.
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INTRODUCTION

Suicide can be a devastating end point of psychiatric disease and is the tenth leading cause of death in the United States (Xu *et al,* 2016). Dopamine signaling critically modulates motivational and emotional processing as well as reward association, and these are altered in mood disorders and in suicide (Dunlop and Nemeroff, 2007). However, whether striatal dopamine receptor or transporter expression is altered in major depressive disorder (MDD) and/or suicide is unclear.

The dorsal striatum, which includes the caudate nucleus and putamen, is the convergent target of glutamatergic cortical and thalamic inputs whose activity is modulated by dopaminergic efferents from the substantia nigra, and is comprised predominantly of GABAergic medium spiny neurons (MSNs) (Beckstead *et al*, 1979; Gerfen, 1985). Dopamine D1 and D2 receptors are expressed primarily postsynaptically on dendritic shafts and spines of distinct subgroups of MSNs (Shen *et al*, 2008; Thibault *et al*, 2013), whereas the dopamine transporter (DAT) is localized chiefly on nigrostriatal axon terminals (Torres *et al*, 2003).

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While some studies report altered indices of dopaminergic signaling in mood disorders and suicide (Dougherty *et al*, 2006; Laasonen-Balk *et al*, 1999; Pitchot *et al*, 2001; Ruiz *et al*, 1992), others find unaltered striatal dopamine receptor binding sites in depression and/or suicide (Bowden *et al*, 1997; Suhara *et al*, 1992); for review, see Savitz and Drevets (2013). The present study, therefore, was designed to test the primary hypothesis that dopamine receptor and/or DAT expression is altered in suicide.

Striatal dopamine may also be disrupted by environmental factors that are implicated in suicide, such as early life adversity (ELA) (Bruffaerts *et al*, 2010). Striatal dopamine release is related to low parental care and increased cortisol levels in response to a psychosocial stressor in humans (Pruessner *et al*, 2004) while in rodent models, maternal separation induces changes in striatal DAT levels (Meaney *et al*, 2002). Behaviorally, ELA is correlated with increased risk of substance abuse, mood disorders, and suicide (Enoch, 2011; Johnson *et al*, 2002; MacMillan *et al*, 2001; Molnar *et al*, 2001). However, the neurobiological effect of ELA on striatal dopaminergic signaling in humans has not yet been reported in the context of suicide.

The current study utilized quantitative autoradiography of coronal sections through the dorsal striatum of suicides and non-psychiatric controls to quantify DAT, D1, and D2 receptor binding *postmortem*. [^{3}H]mazindol was used to label DAT, [^{3}H]-SCH23390 the D1 receptor, and [^{3}H] sulpiride the D2 receptor. Reports of ELA and psychiatric diagnoses were collected for all subjects at psychological autopsy.

MATERIALS AND METHODS

Subjects

Brain tissue was provided by the Allegheny County coroner (Pennsylvania) or the New York City medical examiner offices in accordance with city and county regulations. Protocols were approved by the Institutional Review Board for biomedical research of the New York State Psychiatric Institute or the University of Pittsburgh. The coroner or medical examiner determined the cause of death.

A psychological autopsy was conducted by interviewing at least one informant per case as described by Kelly and Mann (1996) and Mann (2000). The clinical diagnoses were determined using the SCID and DSM-IV criteria, and were confirmed at a consensus conference with the psychologist raters and a group of investigators, including psychiatrists. An Axis I diagnosis of MDD was present in 11 of the 17 suicides. Medications prescribed 3 months antemortem were reported at psychological autopsy. All control subjects underwent a similar psychological autopsy and were psychiatrically normal at the time of death although three of the controls had a lifetime diagnosis (Table 1). The presence of ELA was determined at psychological autopsy. ELA was defined as the occurrence of one or more of the following events before the age of 15: obstetric complications or birth problems, separation from either parent for more than 6 consecutive months, separation or divorce of the deceased parents, having been adopted, having lived in a foster home or orphanage, or a history of physical and/or sexual abuse. ELA was present in 6 of the control subjects and 10 of the suicides. An affirmative response was required to be placed in the ELA category, and subjects were not placed in this category in absence of this information.

Each subject underwent neuropathological examination and toxicological screening. Drug status at the time of death was determined by analysis of peripheral fluids (blood/urine or vitreous) and brain. Individuals with a history of HIV, stroke, central nervous system disease, or cerebral trauma were excluded from the study.

Brains were collected at autopsy. The right hemisphere of each brain was sectioned into 1.5-cm coronal blocks that were immersed in liquid Freon-12 (−20 °C, dichlorodifluoromethane, Suva) while lying flat on a glass slide, and then stored at −80 °C before sectioning. Sixty frozen 20 μm coronal sections at the level of the ventral striatum were collected serially using a large format cryostat (Cryopolycut, Leica, Germany) and thaw-mounted onto acid-cleaned, gelatin-subbed 3" X 5" glass slides. Slides were desiccated and stored at −20 °C for 24 h and −80 °C thereafter. Nissl substance staining was performed on one section every 200 μm. Brain sections were coded and all further processing was conducted by personnel masked to cause of death.

Control and suicide subjects were matched for age (controls: 45.3 ± 21.1 years, suicide: 44.7 ± 22.8 years), sex (14 males and 4 females in each group), and as closely as possible for postmortem interval (PMI; controls: 15.8 ± 5.4 h, suicides: 19.7 ± 4.1 h). Although PMI was significantly longer for suicides ($f = 2.5$, $p < 0.05$), average brain pH did not significantly differ between suicides (pH = 6.63 ± 2.7) and controls (pH = 6.51 ± 0.19). Individual subject data and causes of death are listed in Table 1.

Quantitative Autoradiography of [^{3}H] SCH23390

[^{3}H] SCH23390 was used to quantitate dopamine D1 receptor binding as described by Przedborski *et al* (1991). Sections were placed in a desiccation chamber for 30 min at room temperature before pre-incubation for 30 min at 21 °C in 50 mM Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Sections were then incubated in the same buffer at 23 °C for 90 min with 2 nM [^{3}H]SCH23390 and 50 nM ketanserin tartrate (Sigma-Aldrich, St, Louis, MO) to mask binding to serotonin 2A receptors. Non-specific binding was determined in near adjacent sections with 10 μM cis-(z)-flupentixol dihydrochloride (RBI, MA). Following radioligand incubation, sections were washed twice for 10 min in ice-cold incubation buffer and then briefly dipped in ice-cold deionized water. Sections were then quickly dried and transferred to a vacuum desiccator until exposure.

Dried slides were then assembled in X-ray film cassettes and exposed to tritium-sensitive film (Hyperfilm; Amersham, Uppsala, Sweden) with tritium standards (American Radiolabeled Chemicals, St. Louis, MO) for 5 weeks. Cassettes containing films and sections were stored at 4 °C in a box containing desiccant throughout the period of exposure.

Quantitative Autoradiography of [^{3}H]mazindol

Autoradiography of [^{3}H]mazindol (23 Ci/mmol; NEN, MA) to dopamine reuptake sites in the striatum (Przedborski *et al*, 1991) was performed as described above, with the minor modifications as follows. Briefly, sections were pre-incubated in 50 mM Tris HCl buffer (pH 7.9) containing 120 mM NaCl and 5 mM KCl for 30 min at room temperature. Sections were then incubated in the same buffer with 4 nM [^{3}H] mazindol for 60 min at 4 °C. Non-specific binding was determined using 100 μm nomifensin. Sections were then washed and desiccated, and exposed with tritium standards to Hyperfilm (Amersham) for 4 weeks before developing.

Quantitative Autoradiography of [^{3}H]sulpiride

Autoradiography of D2 receptor binding sites was performed using [^{3}H]sulpiride (70 Ci/mmol: NEN) as described by Jastrow *et al* (1984), with minor modifications. The D2 receptor has up to a 100-fold greater selectivity for sulpiride than the D3 receptor (Vallone *et al*, 2000). Sections were pre-incubated at room temperature for 30 min in 50 mM Tris-HCl buffer (pH 7.7) containing 120 mM NaCl and 5 mM KCl before incubation in the same buffer with 5 nM [^{3}H]sulpiride and 0.1% ascorbic acid for 1 h at room temperature. Non-specific binding was determined using 1 μM Butaclamol (RBI). Following washes and desiccation, slides were exposed to Hyperfilm (Amersham) for 12 weeks.

Radioligand Binding Analysis

Regionally specific receptor binding density was analyzed using imaging software (MCID Elite version 7, Imaging Research, Canada, now Interfocus Imaging, UK). Images were acquired using a 12-bit digital CCD camera mounted on a light stand containing a wide-area diffused light source.

Table 1 Summary of Postmortem Cases

Pair	Group	Axis I diagnosis	Axis 2 diagnosis	Childhood adversity	Age	Sex	Race	Manner of death	Brain toxicology	Blood toxicology	Prescribed[a]	PMI	pH	DAT[b]	D1[b]	D2[b]
1	Control	Alcohol abuse, ADHD	None	Yes	16	Male	African American	Motor vehicle accident, passenger	Barbiturates	None	None	9	6.5	×	×	
	Suicide	MDD, Conduct disorder, ADHD, Marijuana abuse	None	Yes	13	Male	White	Ingestion of bleach	None	None	None	18	6.4	×	×	
2	Control	Alcohol abuse, Conduct disorder	None	Yes	18	Male	African American	Stabbing (homicide)	None	None	None	13	6.5	×	×	×
	Suicide	MDD	Avoidant personality disorder, obsessive/ compulsive	Yes	17	Male	White	Gunshot wound	None	None	None	15	6.8	×	×	×
3	Control	None	None	Yes	18	Male	Hispanic	Cardiovascular	None	None	None	14	6.7	×	×	×
	Suicide	None	Antisocial personality disorder	Yes	18	Male	Hispanic	Hanging	None	Marijuana	None	15	6.8	×	×	×
4	Control	None	None	No	24	Male	Hispanic	Pulmonary embolism	None	None	None	12	6.6	×	×	×
	Suicide	Bipolar disorder, Marijuana abuse	Borderline personality disorder	Yes	24	Male	White	Drowning	Lidocaine	Lidocaine, Lithium	Lithium, Haloperidol	21	5.9	×	×	×
5	Control	None	None	No	43	Male	White	Cardiovascular	None	None	None	23	6.1	×	×	
	Suicide	Schizophrenia	None	No	41	Male	White	Fall from height	None	None	Haloperidol	22	6.4	×	×	
6	Control	None	None	No	66	Male	White	Cardiovascular	None	None	None	19	6.5	×	×	×
	Suicide	None	Schizoid personality disorder	No	63	Male	Hispanic	Hanging	None	None	None	17	6.7	×	×	×
7	Control	Past cocaine abuse	Borderline personality disorder	Yes	25	Male	White	Accidental carbon monoxide inhalation	Cocaine	Carbon monoxide	None	18	6.7	×	×	×
	Suicide	MDD	Avoidant personality disorder	Yes	19	Male	African American	Gunshot wound	None	None	None	24	6.9	×	×	×
8	Control	None	None	No	30	Female	African American	Cardiovascular	None	None	None	8	6.4	×	×	
	Suicide	Schizoaffective disorder, past alcohol, cocaine, and marijuana abuse	None	Yes	26	Female	Hispanic	Hanging	None	None	None	18	6.9	×	×	
9	Control	None	None	No	73	Male	White	CNS	None	None	None	11	6.2			×
	Suicide	MDD	Other	No	77	Male	White	Gunshot wound	None	None	None	13	.			×
10	Control	None	None	Yes	37	Male	African American	Cardiovascular	None	None	None	15	6.8	×	×	×
	Suicide	MDD	Obsessive/ compulsive	Yes	40	Male	White	Hanging	None	Acetaminophen	Zolpidem, Sertraline	20	6.8	×	×	×
11	Control	None	None	No	58	Male	White	Cardiovascular	Lidocaine	Lidocaine	None	22	6.6	×	×	×
	Suicide	MDD	None	No	62	Male	White	Drowning	None	None	Lorazepam	21	6.4	×	×	×

Table 1 Continued

Pair	Group	Axis 1 diagnosis	Axis 2 diagnosis	Childhood adversity	Age	Sex	Race	Manner of death	Brain toxicology	Blood toxicology	Prescribed[a]	PMI	pH	DAT[b]	D1[b]	D2[b]
12	Control	None	None	No	56	Male	Hispanic	Cardiovascular	None	None	None	22	6.4	×	×	×
	Suicide	MDD, Gambling disorder, Obsessive/ compulsive disorder	None	Yes	53	Male	Hispanic	Hanging	None	None	None	27	6.7	×	×	×
13	Control	None	None	No	66	Male	African American	Cardiovascular	None	Anti-hypertensive	None	24	.	×	×	×
	Suicide	MDD, Alzheimer's	None	Yes	71	Male	White	Fall from height	None	None	None	23	6.9	×	×	×
14	Control	None	None	No	45	Female	White	Cardiovascular	None	None	None	22	6.7	×	×	×
	Suicide	MDD	None	No	43	Female	White	Overdose[c]	Acetaminophen, anti-depressants, fluoxetine	Benzodiazepines, opiates, acetaminophen, anti-depressants, fluoxetine	None	12	6.7	×	×	×
15	Control	None	None	Yes	41	Female	African American	Cardiovascular	None	None	None	8	6.6	×	×	×
	Suicide	MDD	None	No	35	Female	White	Hanging	None	Anxiolytics	Alprazolam	19	6.7	×	×	×
16	Control	None	None	No	60	Male	White	Cardiovascular	None	None	None	16	6.6	×	×	×
	Suicide	MDD	None	Yes	59	Male	Hispanic	Hanging	None	None	None	25	6.8	×	×	×
17	Control	None	None	No	89	Male	White	Motor vehicle accident, pedestrian	None	None	None	11	6.4	×	×	×
	Suicide	Delusional disorder	None	No	90	Male	White	Hanging	None	Opiates	None	25	6.8	×	×	

Abbreviations: ADHD, attention deficit hyperactivity disorder; ELA, early life adversity; MDD, major depressive disorder; PMI, postmortem interval.
[a]Prescribed 3 months antemortem, as reported by the next of kin.
[b]Indicates which binding assays were conducted: DAT = [^{3}H]mazindol, D1 = [^{3}H]SCH23390, D2 = [^{3}H]sulpiride.
[c]Combined drug overdose of acetaminophen, propoxyphene, and benzodiazepines.

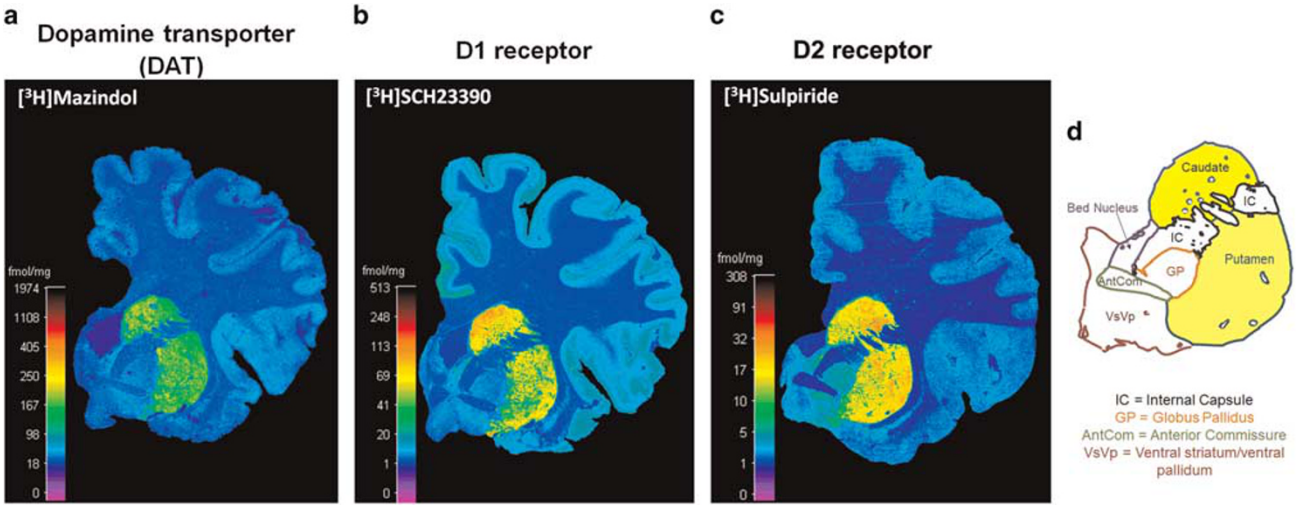

Figure 1 Representative heat-mapped autoradiographs and regions sampled. (a) DAT binding was quantified using [^{3}H]mazindol, (b) D1 receptor binding with [^{3}H]SCH23390, and (c) D2 binding with [^{3}H]sulpiride. Scale bars are in fmol/mg. (d) Regions sampled are outlined. The caudate and putamen (highlighted in yellow) comprises the dorsal striatum, which was analyzed in this study.

The camera output was first digitally normalized to a flat field of illumination. The output was also calibrated for absolute area measurements (in mm^2). The radioisotope standard (ART-123, American Radiolabeled Chemicals), which was exposed to the same film as the sections, was also sampled. The sampled values, in relative optical density (ROD) units, were plotted against the known tissue equivalents of the standards values (in fmol/mg tissue) and a curve was fitted to the values, providing quantitation of the receptor density in units of fmol/mg tissue for sampled anatomical regions of interest. Sectors incubated for total binding and for non-specific binding were imaged and aligned with each other in separate image channels. Regions of interest were then defined as a contour on the total binding image channel.

Sampling the total binding image region simultaneously sampled both total and non-specific channels within the same contour, providing an area-weighted mean of specific ligand binding that is anatomically registered (total − non-specific = specific binding at that region). The caudate nucleus, putamen, internal capsule, bed nucleus of the stria terminalis, globus pallidus, anterior commissure, and ventral striatum/ventral pallidum were then identified, outlined by hand (as illustrated in Figure 1), and sampled. The identification of the region and the sample contour were verified by a second anatomist. Image analysis was conducted by investigators blind to group assignment. SPSS computer software (IBM) was used for statistical analysis of radioligand binding and linear regression analyses. Tissue was not always available for ventral striatal regions, and, therefore, for this study, only data from the dorsal striatal regions (caudate and putamen, highlighted in yellow in Figure 1) were included in the data analysis. Dorsal striatal tissue availability for each individual subject is shown in Table 1.

Statistical Analyses

All statistical comparisons, except correlation coefficient comparisons, were made using untransformed raw data. Comparisons of mean DAT, D1, and D2 receptor binding were carried out using unpaired *t*-tests with Welch's correction. *P*-values and Welch-corrected *t*-values are reported. Statistical significance was determined at $P<0.05$. To determine the relationships between dopamine receptor binding in suicides and controls and to test the hypotheses that dopamine binding is correlated with age or ELA, linear regression analyses were carried out. R^2 and *P*-values are reported. A Fisher's r-to-z transformation was used to calculate a *z*-value to measure the significance of the difference between correlation coefficients.

RESULTS

DAT, D1, and D2 Receptor Binding Does Not Differ Between Suicides and Controls in the Dorsal Striatum

No significant differences in [^{3}H]SCH23390, [^{3}H]mazindol, or [^{3}H]sulpiride binding were observed between suicides and matched controls (Figures 2a and c). Mean dorsal striatal binding of the DAT ligand [^{3}H]mazindol binding was 100.0 ± 26.6 fmol/mg in controls and 99.3 ± 25.6 in suicides ($t=0.07$, $p=0.95$). Mean binding of the D1 antagonist [^{3}H]SCH23390 was 48.9 ± 14.2 fmol/mg in controls and 46.9 ± 14.0 fmol/mg in suicides ($t=0.41$, $p=0.69$), and mean binding of the D2 receptor antagonist [^{3}H]sulpiride was 12.0 ± 4.7 fmol/mg in controls and 12.0 ± 4.0 fmol/mg in suicides ($t=0.02$, $p=0.98$). There were furthermore no mean differences in DAT, D1, or D2 receptor binding between suicides with MDD and controls.

There were also no significant differences in striatal [^{3}H]mazindol, [^{3}H]sulpiride, or [^{3}H]SCH23390 binding between subjects diagnosed with MDD and those who were not. Mean dorsal striatal DAT radioligand [^{3}H]mazindol binding was 100.0 ± 6.0 in non-MDD subjects and 98.83 ± 7.6 in subjects with MDD ($t=0.13$, $p=0.90$). In non-MDD subjects, mean binding of D1 ligand [^{3}H]SCH23390 was 44.51 ± 5.3 and in MDD subjects 49.47 ± 2.7 ($t=0.84$, $p=0.27$). Mean binding of [^{3}H]sulpiride, the D2 receptor radioligand, was 12.66 ± 1.0 in subjects without MDD and

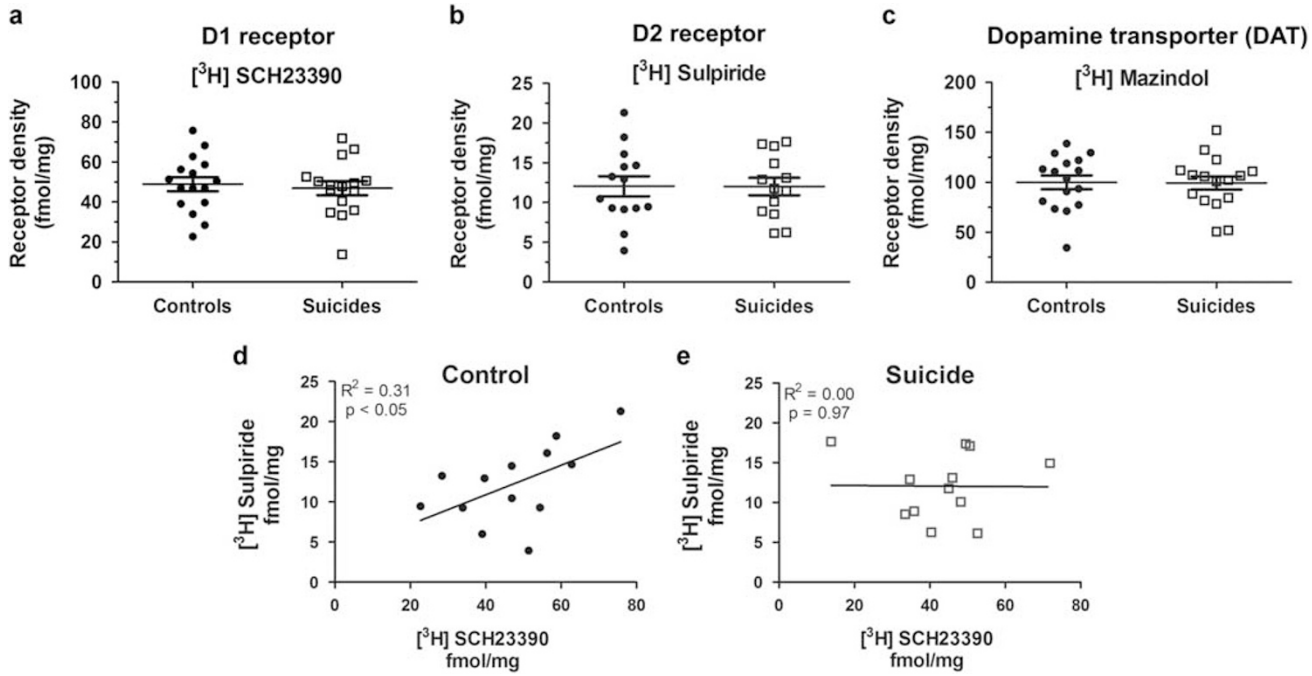

Figure 2 There is no difference in mean striatal D1, D2, and DAT radioligand binding between suicides and controls, but the correlation of striatal D1 and D2 receptor binding levels is disrupted in suicide. (a) D1 antagonist [^{3}H]SCH23390 binding is similar between controls and suicides ($t=0.41$, $p=0.69$), as is (b) D2 antagonist [^{3}H]sulpiride ($t=0.02$, $p=0.98$) and (c) DAT ligand [^{3}H]mazindol ($t=0.07$, $p=0.95$). (d) There is a significant positive correlation ($R^2=0.31$, $p<0.05$) between D1 and D2 receptor binding in control dorsal striatum that does not exist in (e) the dorsal striatum of suicides ($R^2=0.00$, $p=0.97$).

11.65 ± 1.2 in subjects with MDD ($t=0.65$, $p=0.52$). No sex differences were observed in binding.

D1 Receptor and D2 Receptor Binding Is Positively Correlated in Controls But Not in Suicides

To test the hypothesis that, although mean receptor binding is unchanged between suicides and controls, an imbalance in binding between dopamine D1 and D2 receptors might exist in suicide, a linear regression analysis was carried out between [^{3}H]sulpiride and [^{3}H]SCH23390 binding in individual suicides and controls. A positive correlation was found between [^{3}H]sulpiride and [^{3}H]SCH23390 binding in controls ($R^2=0.31$, $p<0.05$) but not in suicides ($R^2=0.00$, $p=0.97$) (Figures 2d and e). Comparison of the two correlation coefficients showed a trend toward statistical significance ($Z=1.44$, $p=0.07$). This may indicate a dysregulation between dorsal striatal dopamine D1 and D2 receptor expression levels in suicide.

DAT and D1 Receptor Binding in the Dorsal Striatum Decreases with Age

Radioligand binding to DAT and the D1 receptor in the dorsal striatum decreased with increased subject age. There was a negative correlation ($R^2=0.12$, $p<0.05$) between age and [^{3}H]SCH23390 binding across both suicides and controls, indicating loss of striatal density of D1 receptors with age (Figure 3a). Likewise, there was a negative correlation ($R^2=0.36$, $p<0.001$) between age and [^{3}H]mazindol binding, indicating decreased DAT availability with advancing age (Figure 3b). We observed no significant correlation between dorsal striatal [^{3}H]sulpiride binding and age in our cohort.

Dorsal Striatal DAT and D1 Receptor Binding Is Positively Correlated in Subjects Who Did Not Experience ELA

ELA was reported in 10 suicides and 6 controls (Table 1). Because the *a priori* primary aim of this study was to examine the differences in striatal dopamine indices in suicide and non-suicide deaths, controls were matched to suicides for sex, age, and PMI—rather than ELA and non-ELA subjects being matched for these factors. While there was no significant difference in PMI between ELA and non-ELA subjects, subjects with reported ELA were significantly younger than those without reported ELA. The mean age of subjects who reported ELA was 32.7 ± 18.4 years while the mean age of those who did not report ELA was 56.1 ± 18.4 years ($t=3.82$, $p<0.001$), which indicates that, in our cohort, ELA is more prevalent in younger suicides and controls. Two of the subjects with reports of ELA were females (one suicide and one control).

Because of the effect of aging on D1 and DAT striatal binding (Figure 3), it was therefore necessary to compare age-adjusted radioligand binding between ELA and non-ELA subjects. Age-corrected binding did not significantly differ between ELA and non-ELA subjects for DAT ($F=2.91$, $p=0.10$), the D1 receptor ($F=0.03$, $p=0.87$), or D2 receptors ($F=0.50$, $p=0.49$).

To determine whether there was an imbalance in dopamine D1 and D2 receptor binding and/or dopamine receptor binding and DAT binding similar to that observed

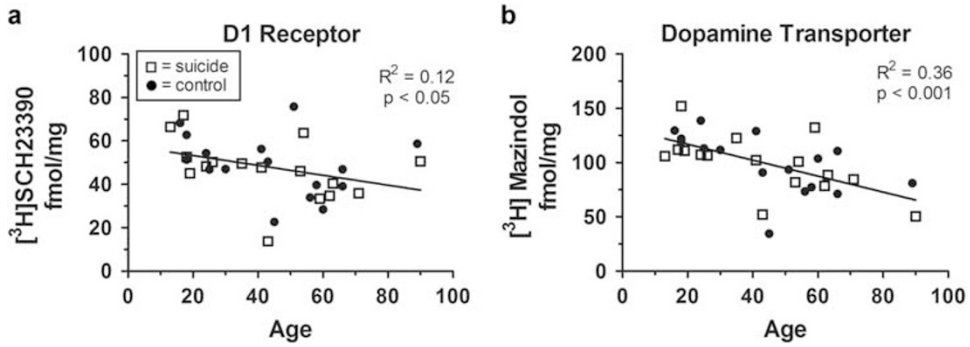

Figure 3 Loss of striatal density of D1 receptors and DAT with age. (a) There is a significant negative correlation ($R^2 = 0.12$, $p < 0.05$) between age and [^{3}H] SCH23390 binding across both suicides and controls. (b) Likewise, there is a significant negative correlation ($R^2 = 0.36$, $p < 0.001$) between age and [^{3}H] mazindol binding, indicating decreased DAT availability with advancing age.

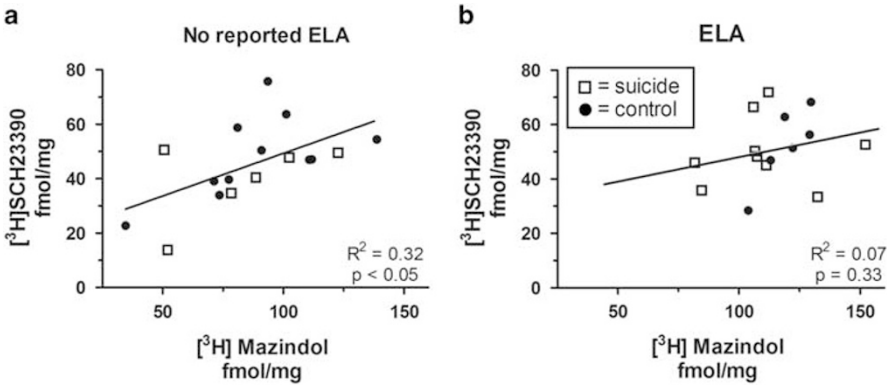

Figure 4 ELA may disrupt the equilibrium between DAT and D1 receptor binding. (a) [^{3}H]mazindol and [^{3}H]SCH23390 binding is positively correlated in subjects without reported ELA ($R^2 = 0.32$, $p < 0.05$) but not in (b) subjects with reported ELA ($R^2 = 0.07$, $p = 0.33$).

in suicide, linear regression analyses were conducted. We found a significant positive correlation between [^{3}H] mazindol and [^{3}H]SCH23390 binding in suicides and controls without report of ELA ($R^2 = 0.32$, $p < 0.05$) that was not observed in subjects with reported ELA ($R^2 = 0.07$, $p = 0.33$) (Figure 4). The difference between correlation coefficients was not statistically significant ($Z = 1.01$; $p = 0.31$); however, this was probably a function of the limited sample size of our study.

DISCUSSION

D1, D2, and DAT radioligand binding was comparable between suicides and controls, and between those with reported ELA and those without known ELA. There was a positive correlation, however, between striatal D1 and D2 receptor binding in controls that was not present in suicides and a similar positive correlation in DAT and D1 receptor binding in non-ELA subjects that was not present in ELA subjects. Across all individuals, a decline in DAT and D1 receptor binding occurred with aging.

The absence of a difference in D1, D2, or DAT radioligand binding in the dorsal striatum of suicides as compared with controls is consistent with previous studies of striatal dopaminergic indices in MDD and suicide (Allard and Norlen, 1997, 2001; Bowden *et al*, 1997; Ryding *et al*, 2006). However, we observed a positive correlation between dopamine D1 and D2 receptor binding in controls that was absent in suicides. A functional correlation between D1 and D2 binding has previously been reported in rodents, suggesting that levels of D1 and D2 binding are normally coordinated, perhaps through a common mechanism, in striatal tissue (Glick *et al*, 1988).

The loss of correlation between D1 and D2 receptor binding in postmortem striatal tissue from suicides might reflect dysregulation of dopamine receptors in suicide, perhaps as a result of previous long-term antidepressant and/or neuroleptic treatment. For example, treatment of adult male rats with antidepressant medications induces changes in D2 receptor mRNA and binding site density in the dorsolateral striatum, but does not change D1 mRNA or binding density (Ainsworth *et al*, 1998).

Alternatively, the dissociation of dopamine D1 and D2 receptor binding levels that we observe in suicide and MDD may reflect a decreased ability to dynamically regulate D1 and D2 receptor availability and a reduced functional link between the two receptors, similar to that reported by Seeman *et al* (1989) in human postmortem striatal tissue from schizophrenia and Huntington's disease. On the cellular level, an imbalance in D1 and D2 receptor availability in suicide brains could influence local and distal synaptic

signaling mechanisms (Calabresi *et al*, 1992; Surmeier *et al*, 2007), while on the level of the individual, a dysregulation of D1 and D2 receptor balance in suicide could hold implications for antidepressant therapy (Wong and Liu, 2012). Because the majority of suicides for whom tissue was processed for both D1 and D2 radioligand binding had a diagnosis of MDD (8 of 12; Table 1), it is unclear from the current study whether an imbalance in D1 and D2 binding might be due to a unique diathesis of suicide or might be more widely observed in MDD. Inclusion of MDD subjects who died by means other than suicide would be a valuable addition for future studies.

This imbalance of dopamine D1 and D2 receptor binding in suicide could be influenced by effects of ELA on DAT and D1 equilibrium. In rodent models, early life stressors are linked to dysregulation of striatal DAT (Brake *et al*, 2004). Additionally, in humans, individuals with a specific haplotype in the *DAT* gene showed increased susceptibility to PTSD following childhood trauma (Drury *et al*, 2013). The observed imbalance in D1 and DAT levels in ELA-exposed subjects may not only have implications for suicide risk, but also for the developmental psychopathology of substance abuse. ELA increases risk of substance use disorders during adolescence and later life (Andersen and Teicher, 2009), and striatal dopamine has a crucial role in the rewarding pathways involved in the action of drugs of abuse and instigation of addiction (Kalivas and Volkow, 2005).

ELA was reported as more prevalent in younger suicides. ELA was determined by response during psychological autopsy, and decreased frequency of ELA report in older subjects may be due to a failure of significant others of older subjects to recall or know of adversity experienced during the subjects' youth due to the passage of time, or due to stigma of ELA leading it to be less frequently discussed in older subjects. However, it could otherwise indicate that ELA is a potentially lethal factor in vulnerability to early suicide (Johnson *et al*, 2002).

DAT and D1 receptor binding declined with age across suicides and controls. This is consistent with previous findings (Kemppainen *et al*, 2001; Rinne *et al*, 1990; Suhara *et al*, 1991; Volkow *et al*, 1996) and may reflect a normal age-related decline in D1 and DAT expression that is also present in MDD or suicide. Conversely, increased D1 and DAT availability in younger subjects may be one developmental factor that ultimately could contribute to a distinct psychopathology in adolescent suicide and MDD.

However, we observed no age-related decline in D2 binding using [^{3}H]sulpiride. While some studies have demonstrated a decrease in striatal D2 binding with increasing age (Antonini and Leenders, 1993; Pohjalainen *et al*, 1998), which may be tied to age-related cognitive decline (Backman *et al*, 2000), these studies were conducted using raclopride rather than sulpiride. Studies conducted using sulpiride report either no change in striatal binding with advancing age (Memo *et al*, 1980) or a more subtle, subregion-specific age-related decline in caudate D2 binding (Morelli *et al*, 1990). These apparent differences in raclopride and sulpiride binding may in fact represent metabolic differences between *in vivo* and *in vitro* studies, as raclopride is the superior ligand for PET studies due to its greater bioavailability and brain penetration (Farde *et al*, 1988); considerations that are not relevant to *postmortem* work.

The current study provides a quantitative comparison of the density of dopamine markers DAT, D1, and D2 receptors between suicides and controls. We found no differences between suicides and controls in DAT or D1 or D2 binding, but that the balance of striatal D1 and D2 receptor binding is disrupted in suicide, and that this might be influenced by ELA. This may have implications for the etiology of suicide psychopathology or may indicate an imbalance of D1 and D2 receptors induced by ELA and/or antidepressant or antipsychotic treatment.

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