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Research Paper

Are dopamine receptor and transporter changes in Rett syndrome reflected in MeCP2-deficient mice?

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Abstract

In the present study we tested the claim that the dopaminergic dysfunction of Rett Syndrome (RTT) also occurs in Mecp2-deficient mice that serve as a model of the syndrome. We used positron emission tomography (PET) to image dopamine D_2 receptors (D_2R) and transporters (DAT) in women with RTT and in Mecp2-deficient mice, and D_1R and D_2R density was measured in postmortem human tissue by autoradiography. Results showed 1) significantly reduced D_2R density in the striatum of women with RTT compared to control subjects. 2) PET imaging of mouse striatum similarly demonstrated significant reductions in D_2R density of 7-10 week-old hemizygous (Mecp2-null) and heterozygous (HET) mice compared to wild type (WT) mice. With age, the density of D_2R declined in WT mice but not HET mice. 3) In contrast, postmortem autoradiography revealed no group differences in the density of D_1R and D_2R in the caudate and putamen of RTT versus normal control subjects. 4) In humans and in the mouse model, PET revealed only marginal group differences in DAT. The results confirm that dopaminergic dysfunction in RTT is also present in Mecp2-deficient mice and that reductions in D_2R more likely explain the impaired ambulation and progressive rigidity observed rather than alterations in DAT.

**Keywords:** Autoradiography; binding potential; gene mutation; intellectual disability; neurotransmitter; nigrostriatal pathway; postmortem; positron emission tomography; partial volume correction; radioligand
Introduction

Rett Syndrome (RTT) is a developmental disorder with cognitive,motor, sensory, emotional, and autonomic functional impairments (Dunn, 2001; Hagberg, 2002; Naidu et al., 1995; Percy et al., 2010), including stereotyped limb movements, dystonia, dyskinesias, progressive rigidity, and profound intellectual disability (Hagberg, 1989; Humphreys and Barrowman, 2016; Kerr, 1995; Percy et al., 2010; Segawa, 2001). More than 90% of patients with the RTT phenotype have mutations in the MECP2 gene (Bebbington et al., 2008; Hoffbuhr et al., 2001; Percy et al., 2007). The present investigation characterized the expression of dopaminergic D1 and D2 receptors (D1R and D2R), and dopamine transporter (DAT) availability (radioligand binding potentials) in human RTT and in MeCP2-deficient mice. By relating findings in humans to consequences of the gene mutation in mice, we tested the claim that MeCP2-deficient mice qualify as a model of the dopaminergic deficits in human RTT.

Early studies of girls with RTT demonstrated reductions in levels of dopamine (DA) and its metabolite homovanillic acid (HVA) in plasma (Riederer et al., 1985), cerebral spinal fluid (CSF) (Percy et al., 1985; Zoghbi et al., 1989; Zoghbi et al., 1985) and in postmortem brain tissue of patients with RTT (Lekman et al., 1989; Riederer et al., 1986; Wenk, 1995; Wenk et al., 1991), while other studies failed to reveal changes in CSF HVA level (Lekman et al., 1990; Perry et al., 1988). In RTT, levels of tyrosine hydroxylase, the rate limiting enzyme for DA synthesis, also were decreased in the substantia nigra pars compacta (SNpc), the origin of the nigrostriatal pathway (Jellinger et al., 1988; Lekman et al., 1989). Postmortem tissue from girls with RTT showed reduced levels of DA in putamen and cerebral cortex and of choline acetyltransferase in caudate nucleus (Wenk et al., 1991), suggesting significant perturbation of nigrostriatal and basal forebrain pathway activities. While reductions in extracellular DA concentrations normally lead to up-regulation of DA receptors, postmortem brains of three patients with RTT had reduced [3H]spiroperidol binding to the D2R compared to control brains.
The reductions in DA receptors occurred in the context of reductions in the volume of the caudate nuclei and mid-brain regions (Reiss et al., 1993), suggestive of atrophy that can bias determinations of dopamine receptor density.

Mecp2-deficient mice have enabled the examination of possible specific effects of Mecp2 deficiency on neurotransmitter levels. Mecp2-deficient mice have impaired DA function (Gantz et al., 2011; Panayotis et al., 2011a; Panayotis et al., 2011b) and decreased biogenic amine concentrations (Ide et al., 2005; Panayotis et al., 2011a; Viemari et al., 2005), suggesting that the Mecp2 deficiency has significant effects on several neurotransmitter systems, as also seen in patients with RTT.

We compared the densities of D$_2$R and DAT in vivo in women with RTT (versus healthy control subjects) and Mecp2-deficient mice (versus wildtype (WT) mice) to determine 1) whether there was dopaminergic pre- and post-synaptic dysfunction in MeCP2 deficient human and mouse brains, and 2) whether the mouse brain exhibits the same brain pathology observed in humans. We measured D$_2$R density by mapping D$_2$-like DA receptors in vivo in humans and mice (Wong et al., 1986a; Wong et al., 1986b; Wong et al., 1997a; Wong et al., 1997b) and by receptor autoradiography in postmortem human striatum. We also measured dopamine transporter (DAT) density as an index of the number of nigrostriatal terminals in vivo in humans and mice. Together these measures validate the use of mice with Mecp2 deficiency as models for the integrity of dopaminergic neurotransmission in women with RTT.

Materials and Methods

Patients with RTT and healthy volunteers: We included women diagnosed with RTT, based on clinical criteria and mutations in MECP2, and normal age-matched women volunteers. Consent was given by the appropriate family members or legal guardians, or by the women themselves, following the rules of the Johns Hopkins University Investigational Review Board.
Table 1 lists the age, PET scanner, MECP2 mutation status, and primary neurological manifestations for the women with RTT studied. The age of the subjects studied ranged from 15-32 years of age.

**Mice**

The Animal Care and Use Program at Johns Hopkins University approved our mouse study protocol. Genotyping of mice was performed using the DNAeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA), as in our previous publications (Blue et al., 2015; Metcalf et al., 2006). For D_{2}R PET studies, we included 15 MeCP2-heterozygous (HET) and 6 MeCP2-null mice (Adrian Bird model (Guy et al., 2001)) that ranged in age from 7-33 weeks of age. For DAT studies, we included 11 WT, 5 MeCP2-null mice and 6 HET mice that were 7-17 weeks of age. For volumetric studies, we included mice at 4 (6 WT, 7 HET, 8 MeCP2-null), 7 (7 WT, 6 HET, 8 MeCP2-null) and 14 weeks (8 WT, 7 HET, 6 MeCP2-null) of age.

**Positron Emission Tomography (PET)**

Magnetic resonance imaging (MRI) (for humans) and computerized tomography (CT) (for mice) were obtained for purposes of localization and anatomic correlation with subsequent PET imaging studies. PET studies for D_{2}R included two cohorts of patients. The first cohort included 6 women with RTT and 9 controls who had tomography in the CTI NeuroEcat scanner (Siemens CTI, Knoxville, TN, USA). The second cohort of 4 women with RTT and 7 controls later had higher resolution tomography with the GE4096+ whole body PET device that allowed the caudate and putamen to be distinguished. Each participant (healthy or RTT) received 2 PET sessions (baseline and blocked conditions) using 3-N-[\textsuperscript{11}C]methylspiperone ([\textsuperscript{11}C]NMSP) at high specific activity, namely, 74-111GBq/μmole (2-3 Ci/μmole) (Dannals et al., 1986) in order to calculate absolute D_{2}R B_{max}. For the CTI NeuroECAT studies, subjects had up to 12 acquisitions during the 90-minute scans (Wong et al., 1986a; Wong et al., 1986b; Wong et al., 1997a).

To increase the number of subjects in each group, we joined the results of each study
after normalization of data obtained with the different tomographs together. For the second cohort we normalized the values by averaging the values for the caudate and putamen. We then calculated the mean value for each control group in each cohort and expressed the values for each subject as percent control and performed statistical analysis for the two cohorts together.

We used PET to quantify DAT in 9 women with RTT and 8 volunteer control subjects. DAT were labeled with $[^{11}\text{C}]$WIN35,428 with specific activities typically exceeding 74 Gbq/μmole (2 Ci/μmole) (Frost et al., 1993; Wong et al., 1993). All studies used the GE 4096+ tomograph with radial arterial plasma sampling at 50 time-points, where image acquisitions varied from 15 seconds to 6 minutes in duration over a 90-minute total scan period. Partial volume corrections were performed as described previously (Rousset et al., 1998).

The GE mouse PET/CT device (Argus–drT), part of the Small Animal Imaging Resource Program (SAIRP) at the Johns Hopkins University, was used for PET imaging of mice. In striatum, $D_2R$ were labeled with $[^{11}\text{C}]$raclopride (a $D_2/D_3$ antagonist), and DAT were labeled with $[^{11}\text{C}]$methylphenidate ($[^{11}\text{C}]$MP). For all mouse PET data, we estimated the binding potential (BP$_{ND}$) (Gjedde et al., 2005; Innis et al., 2007) with the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996) and plasma reference graphical analysis (LOGAN) (Logan et al., 1996). The two methods have different weaknesses and strengths, suggesting that more or less identical results with both methods would gauge the precision of the findings.

**Postmortem Human Brain Autoradiography**

Postmortem fresh frozen brain samples from 6 girls with RTT (ages 2-25 years; mutation positive) and 10 control females (ages 2-20 years) were obtained from the Harvard Brain Tissue Resource Center (McLean Hospital) and from the Brain and Tissue Banks for Developmental Disorders (University of Maryland). Age and postmortem interval and the $MECP2$ mutation status are listed in Table 2; the mean age range for each group did not differ significantly. Blocks containing the caudate and putamen from the postmortem samples were cut at 20 μm
thick on a cryostat; adjacent coronal sections were processed for autoradiographic labeling of D₁R and D₂R. D₁R binding sites were labeled with 4 nM [³H]SCH23390 in a 50 mM Tris-HCl (pH 7.4) buffer with 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂ and 1 mM MgCl₂ with 1mM ketanserin as a displacer for serotonergic receptors. Nonspecific binding was determined using 1 mM cis (z) flupenthixol (RBI) (Goldman-Rakic et al., 1990). D₂R binding sites were labeled using 2 nM [³H]raclopride in a 50 mM Tris-HCl buffer with 150 mM NaCl (pH 7.4), 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 0.1% ascorbic acid (Goldman-Rakic et al., 1990). Nonspecific binding was determined with 1 mM (+) butaclomol (RBI). Autoradiographically labeled sections were apposed to Amersham [³H]tritium sensitive Hyperfilm for 1-4 weeks, developed photographically, and the films were analyzed using a video based image analysis system as described previously (Blue et al., 1999a). In every section analyzed, the caudate or putamen was outlined and the density (B_max) within each region was measured. Mean densities for every case were derived from the analysis of 3-4 sections. Unfortunately we were not able to measure binding to D₁ and D₂R for every region in every subject.

**Mouse Brain Volumetry**

We measured the volume of striatum in mouse brain. At each age, mice were anesthetized and perfused and the brains removed and stored as described previously (Metcalf et al., 2006; Mullaney et al., 2004). Sections were cut in the sagittal plane (40 μm), mounted on slides and stained with cresyl violet (Nissl stain). Using Steroinvestigator image analysis software, every 4th section (160 μm apart) of striatum was outlined. Total volume of the striatum was determined using Cavalieri’s method.

**Statistical analyses**

Prism GraphPad was used to perform statistical analyses. For human analyses, the densities of D₂R and DAT in RTT versus controls were compared by t-tests and changes with age were evaluated by a linear regression analysis. For DAT, the effects of partial volume
corrections on density were evaluated by 2-way analyses of variance (ANOVA). For mouse studies, 1-way ANOVAs were used to compare \( \text{D}_2\text{R} \) densities in HET, \textit{Mecp2}-null and WT mice that were <11 weeks of age and \( t \)-tests were used for older ages when only HET and WT mice were compared. For both mouse and human studies, linear regression analyses were used to evaluate whether density changed with age. In the case of the DAT binding in mice results, one value for a WT mouse and one value for a HET mouse were excluded as outliers based on criteria for values \( \pm 2 \) standard deviations from the mean.

\section*{Results}

\textbf{PET of Human}

\( \text{D}_2\text{R} \): We estimated the density of \( \text{D}_2\text{R} \) \( \left( \text{B}_{\text{max}} \right) \) in brain by PET with a CTI NeuroEcat scanner after administration of 3-\( N \)-\( [^{11}\text{C}] \)methylpiperone (\( [^{11}\text{C}]\text{NMSP} \)) to 6 women with RTT and 9 healthy adults (age-matched controls), using the previously validated methods of Wong et al. (Wong et al., 1986a; Wong et al., 1986b; Wong et al., 1997a; Wong et al., 1997c). Another cohort of patients with RTT (n=4) and age-matched control subjects (n=7) were imaged with a GE 4096+ PET scanner. The results showed that the normalized \( \text{B}_{\text{max}} \) of \( \text{D}_2\text{R} \) was significantly lower in women with RTT compared to controls (Fig 1A; \( p<0.05 \)). A linear regression analysis did not show a significant change in \( \text{B}_{\text{max}} \) of \( \text{D}_2\text{R} \) with age for either group (\( p=0.330 \) for controls; \( p=0.104 \) for RTT; Fig 1B). However there was a trend for the slopes for the two lines to be different from one another (\( p=0.09 \)) and averages were significantly different (\( p<0.05 \)). These results indicate that there are not significant age-related changes in the density of \( \text{D}_2\text{R} \), at least in the age range of the subjects in our study (15-32 years).

\textbf{In vivo PET of \textit{D}_2\textit{R} in mice}

PET images of \( [^{11}\text{C}]\text{raclopride} \) binding that labeled \( \text{D}_2\text{R} \) in 7-10 weeks old \textit{Mecp2}-deficient mice showed significantly decreased densities of \( \text{D}_2\text{R} \) compared to WT mice (\( p<0.01 \);
Fig 2A, 2B). D2R BP<sub>ND</sub> values were significantly reduced in HET mice and in *Mecp2*-null mice compared to WT mice (**p<0.01 for SRTM analysis, Fig 2A; *p<0.05 for Logan analysis, Fig 2B). PET was not feasible in *Mecp2*-null mice after 8-9 weeks due to decline of health and ultimate death between 10-15 weeks of age. D2R BP<sub>ND</sub> decreased significantly with age in WT mice (Linear regression analysis p<0.001; Fig 2C (SRTM) and Fig 2D (LOGAN)) but not in HET mice. As a result, D2 receptor densities were not significantly different in HET and WT mice that were 15 or more weeks of age (Fig 2A, 2B).

**Postmortem autoradiography of D1 and D2R in humans**

No group differences in density of D1R or D2R were observed in postmortem caudate or putamen (Fig 3). However, B<sub>max</sub> for D1R was higher than for D2R in caudate nucleus (p<0.01) and putamen (p<0.05). Linear regression analyses showed no age related changes in D1R density. D2R B<sub>max</sub> in the putamen did decrease with age in both groups (p<0.05).

**In vivo PET of DAT in humans**

To estimate the density of DAT in caudate nucleus and putamen, we obtained PET images of [11C]WIN35,428 uptake and binding in the brains of 9 women with RTT and 8 control subjects with the GE 4096+ tomograph. In the caudate of the RTT, DAT BP<sub>ND</sub> values were lower than in the control subjects (p<0.05; t-test). However, partial volume correction eliminated all significant group differences of DAT density (Fig. 4).

Two women, with 502T/R168X (*) and C397T/R133C (+) mutations, respectively, completed PET sessions with both D2R and DAT (Table 1). Comparing the data for D2R and DAT (partial volume corrected values) for each patient with RTT to the means for the controls revealed that the patient with R168X mutation had lower than normal DAT (68% and 47% of the mean for caudate and putamen respectively) and D2R densities (44% of the mean). The patient with R133C mutation had lower than normal DAT values in the caudate (49% of the mean for Controls) but normal DAT (100.01% of the Control mean) in the putamen. Low normal densities
of D<sub>2</sub>R were observed in the caudate (90% of the mean value).

**In vivo PET of DAT in Mecp2-deficient mice**

PET of [<sup>11</sup>C]methylphenidate ([<sup>11</sup>C]MP) showed a decreased BP<sub>ND</sub> in Mecp2-null mice but the significance varied depending on the type of analysis. (1-way ANOVA, SRTM-p<0.05, Fig 5A; 1-way ANOVA, LOGAN-not significant, Fig 5B). For SRTM analysis, no significant changes in DAT BP<sub>ND</sub> with age were found for the three genotypes (Fig 5C), although regression analysis did show trends for DAT binding to increase with age in WT mice (p=0.07) and for the regression line slopes to be different (p=0.11). The elevations of the intercepts were significantly different (p<0.05) indicating that the regressions lines for HET, Mecp2-null and WT mice were different from one another. For the LOGAN analysis, regression analysis did show significant increases for DAT BP<sub>ND</sub> in WT mice (p<0.05), and regression line slopes for the genotypes were different (p<0.05; Fig 5D).

**Volumetry of mouse striatum**

We measured the volume of the striatum in separate groups of mice that were 4, 7, and 14 weeks of age. The results of one-way ANOVA at different ages showed that the volume of the striatum was lower in Mecp2-null mice than in WT mice at 7 and 14 weeks of age (p<0.01; Fig 6), and compared to HET mice at 14 weeks (p<0.0001). Volume of the striatum in Mecp2-null mice did not differ significantly among the three age groups, while volume in WT mice significantly increased between 4 and 7 weeks (p<0.001) and for HET mice between 7 and 14 weeks of age (p<0.01).

**Discussion**

The present in vivo PET findings of receptor binding are summarized in Table 3. The most significant findings were that PET studies revealed declines in binding to D<sub>2</sub>R and more variable effects on binding to DAT in women with RTT and in Mecp2-deficient mice. The similarities between the results for mice and human PET studies indicate that the Mecp2-
deficient mice serve as an adequate model for dopaminergic deficits in RTT.

**D₁R and D₂R**

Stereotyped hand-wringing, progressive rigidity, dyskinesia, and dystonia are symptoms found in RTT that are thought to reflect perturbed dopaminergic neurotransmission (Chiron et al., 1993; Dunn, 2001; Humphreys and Barrowman, 2016; Jellinger et al., 1988; Segawa, 2005). The present human and mouse PET findings demonstrated reduced D₂R numbers in striatum of females with RTT and brains of Mecp2-deficient mice. Significant age related changes in D₂ receptors were observed in Mecp2-deficient mice but not in our human PET imaging study. The present human PET results differ from those of a single photon emission computed tomography (SPECT) study that demonstrated increased D₂R numbers (Chiron et al., 1993). In addition to the higher spatial resolution of the PET studies and the determination of absolute density (Bₘₐₓ), the age ranges were different in the two studies: patients with RTT aged 4-15 years in the SPECT study and patients with RTT aged 15-30 years in the present study. These findings suggest age-related changes in D₂R such that patients may have higher densities than normal in the first decade of life but lower densities as they approach adulthood. This speculation underlines the importance of characterizing age-related differences of brain development in RTT (Blue et al., 1999b) as well as the use of high resolution PET imaging technology.

In contrast to *in vivo* PET results, human postmortem quantifications of D₁R and D₂R did not reveal any differences in Bₘₐₓ values between patients with RTT and age-matched control subjects. The lack of differences in D₁R and D₂R densities in postmortem human tissue subjected to autoradiography could be explained by the different age ranges in the two studies (15-28 versus 2-25), or issues with postmortem degeneration, or by methodological differences (PET vs. autoradiography). For example, PET can measure endogenous DA changes and other *in vivo* synaptic differences while autoradiography cannot (Wong and Gjedde, 1996). Each of these factors likely contributed to the differing results obtained. We did not perform
autoradiographic studies in the mouse model for RTT given the concordance of the findings for

in vivo PET imaging studies in humans with RTT and Mecp2-deficient mice.

**DAT**

The number of re-uptake sites of dopamine generally was preserved both in caudate nucleus and putamen in women with RTT, compared to control subjects. While the observed values for DAT BP\textsubscript{ND} were significantly reduced in the caudate nucleus of women with RTT compared to control subjects (p<0.05), this difference was lost after partial volume correction suggesting the reduction was not as robust as the D\textsubscript{2}R findings. In mice, the effects of Mecp2-deficiency on DAT varied by the type of analysis model used. While the SRTM method showed a significant decrease in the BD\textsubscript{ND} in Mecp2-null mice compared to WT, the LOGAN analysis model found significant age related changes in BD\textsubscript{ND} in WT mice that were not observed in Mecp2-deficient mice. The finding that the two methods gave different results suggests that DAT numbers were less affected by Mecp2 deficiency than D\textsubscript{2}R numbers where both methods showed similar results.

Past studies reporting dopaminergic dysfunction in RTT provide somewhat conflicting results. Wenk examined D\textsubscript{1}R and DAT in both younger and older patients with RTT and found that the number of D\textsubscript{1}R in the caudate and the density of DAT in the cingulate and midfrontal cortices were unchanged compared to controls, while DAT numbers were decreased in the caudate and putamen (Wenk, 1995). Considering the present findings together with the studies of others, a parsimonious explanation would be that younger patients with RTT have normal-to-above normal dopaminergic activity, but as the patients age, a steady developmental dopaminergic imbalance may unfold, contributing to the clinical features of increased muscle tone and rigidity seen in this disease.

Findings from previous studies of amino acid receptors in postmortem human tissue revealed greater age and group differences than found for the dopamine receptors. Glutamate
receptor expression was not different in the basal ganglia of younger patients with RTT (<10 years), but NMDA and AMPA receptor densities were lower than in age-matched controls in the putamen of older (>10 years) patients with RTT (Blue et al., 1999a). In contrast, GABA receptor density was increased in the caudate nucleus of young patients with RTT (Blue et al., 1999a). There are noteworthy and direct interactions between dopamine and glutamate receptors (reviewed in (Wang et al., 2012)) and it is reported that loss of DA leads to loss of glutamatergic synapses and dendritic spines on striatal neurons by removing the D2R brake on somato-dendritic excitability via activation of L-type calcium channels (Wang et al., 2012). Thus, the alterations in DA and glutamate receptor expression could mediate both the morphological changes in the basal ganglia (atrophy) and the functional modifications of locomotor activity observed in RTT. The present data indicates that the movement disorders seen in girls with RTT syndrome are more likely to reflect secondary physiological effects on dopamine neurons of perturbed receptors of glutamate and GABA than primary degenerative changes in dopaminergic synapses.
Tables and Figures

**Table 1. Clinical data for human patients in PET imaging study.** Clinical data about females with Rett syndrome (RTT) who underwent PET scans to estimate the density of D2 dopamine receptors (D2R) and of dopamine transporters (DAT) in the striatum. Two women indicated by (‘) and (‘) underwent both procedures. Abbreviations: Rigid=rigidity; Trem=tremor; val=valproate; carb=carbamazepine; top=topiramate; phen=phenobarbital.

**Table 2. General information on postmortem samples.** The table lists the case numbers given to the brain tissue samples from University of Maryland (controls and #448) and Harvard Brain Banks, the age at time of death, the postmortem interval (PMI), the group and the specific MECP2 mutation for each RTT case.

**Table 3. Data summary.** Summary of changes in D2R and DAT expression in humans with RTT and in Mecp2-deficient mice.

**Fig 1. D2R density is decreased in RTT.** PET studies showed that binding of 3-N-[11C]methylspiperone to D2R was lower in women with RTT (n=10) compared to age-matched controls (n=16; *p<0.05, 1A). An age versus density plot of densities for each individual showed a trend for D2R densities to decrease with age in controls but not in women with RTT (1B).

**Fig 2: Mecp2-deficient mice have decreased D2R densities.** PET imaging of D2R labeled with [11C]raclopride in the Bird mouse model of Mecp2 deficiency showed that at 7-10 weeks of age, Mecp2-heterozygous (HET) and Mecp2-null mice had significantly lower D2R BP$_{ND}$ values than in wildtype (WT) mice (**p<0.01 for SRTM analysis, 2A; *p<0.05 for Logan analysis, 2B). With age, D2R BP$_{ND}$ declined significantly in WT mice but not in HET mice (p<0.001; 2C}
(SRTM) and 2D (LOGAN)). $D_2R BP_{ND}$ values did not differ significantly between HET and WT mice that were 15 to 33 weeks of age.

**Fig 3: Postmortem studies did not reveal changes in $D_1$ and $D_2R$ density in the striatum of RTT.** Scatter plots of the $D_1$ and $D_2R B_{max}$ (picomoles per mg protein) by age in the caudate (3A and 3B) and putamen (3C and 3D) and regression lines for postmortem brains of patients with RTT (gray open circles and dashed lines) and age-matched controls (black filled squares and solid lines). No group differences were observed in the densities of $D_1R$ and $D_2R$. Linear regression analyses showed that $D_2R B_{max}$ decreased significantly with age in the putamen of control and RTT cases ($p<0.05$ for both).

**Fig 4: Dopamine transporter density is unchanged in RTT.** Densities of $[^{11}C]WIN35,428$ labeled DAT in the caudate and putamen of 9 women with RTT were compared to 8 healthy adults. Both the original observed values and those with the partial volume correction (PVC) are shown. Observed DAT values were significantly less in the caudate of women with RTT compared to controls (t-test; $p<0.05$), but not after partial volume correction.

**Fig 5: SRTM and LOGAN analyses revealed different effects of Mecp2-deficiency on dopamine transporter density in mouse striatum.** SRTM analysis of $[^{11}C]$methylphenidate binding to DAT showed significant reductions in the $BP_{ND}$ for Mecp2-null compared to WT mice (1-way ANOVA, $p<0.05$, Fig 5A) but LOGAN analysis did not (Fig 5B). Using the SRTM model, no age-related changes in the densities of DAT were observed among the three genotypes (Fig 5C). The LOGAN method revealed a significant increase in DAT $BP_{ND}$ for WT mice with age that was not present in Mecp2-deficient mice.
**Fig 6: Volume of the striatum is reduced in Mecp2-null mice.** Unbiased stereological analysis showed that volume of the striatum was less in Mecp2-null mice than in WT mice at 7 weeks (1 way ANOVA, \( **p<0.01 \)). By 14 weeks, Mecp2-null mice had significant reductions in striatal volume compared to WT (\( **p<0.01 \)) and HET (\( ****p<0.0001 \)) mice. With age, the volume of the striatum increased significantly in both WT and HET mice but not in Mecp2-null mice.

**Authorship and Contributorship**

Dean F. Wong, Mary E. Blue, James R. Brašić, Ayon Nandi, Heather Valentine, Kirstie H. Stansfield, Olivier Rousset, Genila Bibat, Mary E. Yablonski, Myron Yaster, Michael V. Johnston, Albert Gjedde and SakkuBai Naidu made 1) substantial contributions to conception and design (SN, DFW, MEB,), acquisition of data (JRB, HV, AN, KHS, MEB, GB, MY, SN), or analysis (MEB, AN, KHS, OR, JRB) and interpretation of data (MEB, JRB, SN, DFW, MVJ); 2) drafting the article or revising it critically for important intellectual content (MEB, AG, JRB, SN, DFW); and 3) final approval of the version to be published (all authors).

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†Deceased
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Conflicts of Interest

No conflicts of interest to report.
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Table 1. Clinical data for Rett patients in study

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<th>Study</th>
<th>Age</th>
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Table 2. General Information on Postmortem Samples.

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Table 3. Results summary

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<td>Mecp2-null, HET&lt;WT at 7-10 weeks of age; Fig. 2A, 2B</td>
<td>RTT=Control; Fig. 3</td>
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<td><strong>D_{2}R Change with Age</strong></td>
<td>No group changes in density with age; Fig. 1B</td>
<td>Density declines with age in WT but not HET; Fig. 2C, 2D</td>
<td>No group changes in density with age; Fig. 3</td>
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<tr>
<td><strong>DAT density</strong></td>
<td>Decrease in RTT caudate disappears with PVC; Fig. 4</td>
<td>Mecp2-null, HET&lt;WT (SRTM); Fig. 5A; density increases in WT (LOGAN); Fig. 5D</td>
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</table>
Highlights

- PET imaging of dopamine function was performed in MeCP2-deficient humans and mice.
- D_2 receptor binding is reduced in Rett syndrome and in Mecp2-deficient mice.
- Dopamine transporters are less affected than D_2 receptors by MeCP2 deficiency.
- Mecp2-deficient mice model dopamine dysfunction in Rett Syndrome.
- D_2 receptors may play a role in motor deficits in Rett Syndrome.
PET imaging to $D_2$ Receptors: Human striatum

A.

![Graph showing the density of $D_2$ receptors in control and Rett groups. The graph includes error bars and indicates a significant difference (*). The control group has $n=16$ while the Rett group has $n=10$.]

B.

![Graph showing the relationship between age and density of $D_2$ receptors for control and Rett groups. The graphs for control and Rett groups are distinguishable by symbols (■ for control, ○ for Rett). The trend lines indicate a positive correlation with age.]

Figure 1
Figure 3

D₁ and D₂ Receptor Binding in Postmortem Striatum

A. D₁: caudate

B. D₂: caudate

C. D₁: putamen

D. D₂: putamen

- Control
- Rett

Bₘₐₓ (pmols/mg protein)

Age (years)
PET imaging to the Dopamine Transporter: Human

Figure 4
Figure 5

PET imaging to the Dopamine Transporter: Mouse striatum

A. SRTM

B. LOGAN

C.

D.

**BP_{ND}**

**Age (weeks)**

WT, HET, Mecp2-null

**n=11, n=6, n=5**

"*" indicates significance.
Figure 6

Striatum volume

- WT
- HET
- Mecp2-null

Volume (mm³)

4 weeks 7 weeks 14 weeks

Figure 6