

Dopamine transporters are markedly reduced in Lesch–Nyhan disease *in vivo*

(mental retardation/positron emission tomography/neurodevelopment/Rett syndrome)

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ABSTRACT Dopamine (DA) deficiency has been implicated in Lesch–Nyhan disease (LND), a genetic disorder that is characterized by hyperuricemia, choreoathetosis, dystonia, and compulsive self-injury. To establish that DA deficiency is present in LND, the ligand WIN-35,428, which binds to DA transporters, was used to estimate the density of DA-containing neurons in the caudate and putamen of six patients with classic LND. Comparisons were made with 10 control subjects and 3 patients with Rett syndrome. Three methods were used to quantify the binding of the DA transporter so that its density could be estimated by a single dynamic positron emission tomography study. These approaches included the caudate- or putamen-to-cerebellum ratio of ligand at 80–90 min postinjection, kinetic analysis of the binding potential [$B_{\max}/(K_d \cdot V_d)$] using the assumption of equal partition coefficients in the striatum and the cerebellum, and graphical analysis of the binding potential. Depending on the method of analysis, a 50–63% reduction of the binding to DA transporters in the caudate, and a 64–75% reduction in the putamen of the LND patients was observed compared to the normal control group. When LND patients were compared to Rett syndrome patients, similar reductions were found in the caudate (53–61%) and putamen (67–72%) in LND patients. Transporter binding in Rett syndrome patients was not significantly different from the normal controls. Finally, volumetric magnetic resonance imaging studies detected a 30% reduction in the caudate volume of LND patients. To ensure that a reduction in the caudate volume would not confound the results, a rigorous partial volume correction of the caudate time activity curve was performed. This correction resulted in an even greater decrease in the caudate-cerebellar ratio in LND patients when contrasted to controls. To our knowledge, these findings provide the first *in vivo* documentation of a dopaminergic reduction in LND and illustrate the role of positron emission tomography imaging in investigating neurodevelopmental disorders.

Several neurodevelopmental disorders are thought to involve the neuropathology of the basal ganglia. Among them, the best known are Lesch–Nyhan disease (LND) and Rett syndrome. In both of these conditions, there is evidence for the dysfunction of the dopaminergic neurotransmission.

LND is an X-chromosome-linked disease with infantile onset characterized by hyperuricemia, choreoathetosis, dystonia, and compulsive self-injury (1). The underlying defect is a near absence of hypoxanthine-guanine phosphoribosyl transferase (HPRT) (2), an enzyme that is normally present in the brain in greater amounts than in other organs. Three lines of

evidence suggest that HPRT deficiency is linked to abnormal dopamine (DA) function in LND: (i) an autopsy study of three LND subjects demonstrated a marked reduction in the DA content and in the activity of DA-synthesizing enzymes in the caudate and putamen (3); (ii) when neonatal rats are depleted of DA with the neurotoxin 6-hydroxydopamine, self-injurious behavior (similar to that seen in LND) occurs when the rats are challenged with 3,4-dihydroxyphenylalanine (L-dopa) as adults (4, 5); and (iii) in an HPRT-deficient mutant mouse strain, there is a reduction of striatal tyrosine hydroxylase and in the number of striatal dopamine transporters measured with ³H-N-[1-(2-benzo(β)thiophenyl) cyclohexyl]piperidine (³H-BTCP) (6). The pertinence of the loss of brain DA in LND has been questioned due to the limited scope of the autopsy study and the fact that the HPRT-deficient mouse study showed only a modest reduction of DA with no self-injury, or other clinical symptoms of LND (6). Therefore the question of dopamine reduction *in vivo* remains unresolved.

The availability of positron emission tomography (PET) ligands that bind to pre- or postsynaptic DA sites has made it possible to study the DA system *in vivo* in patients affected with disorders that involve the basal ganglia. For example, the DA transporter probe β-carbomethoxy-3β-4-fluorophenyl-tropane (CFT; WIN-35,428) (7–11) has been used to detect the degeneration of DA nerve terminals in adult humans (12, 13). It has also been used to measure the degeneration of nerve terminals in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primates and in idiopathic Parkinson disease (14–16). In addition, WIN-35,428 is suitable for evaluating the integrity of DA-containing neurons in neurodevelopmental disorders.

In the present investigation, the hypothesis—that HPRT deficiency in LND is associated with impaired development of dopaminergic projections to the striatum—was tested by determining the number of DA transporters *in vivo* by using PET with the radiolabeled DA transporter ligand WIN-35,428. The measurement of the presynaptic DA sites of WIN-35,428 using PET provides a relevant assessment of the degree of dopaminergic deficiency in LND.

The binding of [¹¹C] WIN-35,428 in LND patients was compared to the binding in healthy volunteers and in patients with Rett syndrome. Subjects with LND or Rett syndrome were studied under anesthesia while the normal controls were not sedated. Rett syndrome was chosen for comparison because of its stereotypical hand-to-mouth movement symptoms that might be linked to the DA system, findings of reduced DA

Abbreviations: LND, Lesch–Nyhan disease; HPRT, hypoxanthine-guanine phosphoribosyl transferase; PET, positron emission tomography; DA, dopamine, L-dopa, 3,4-dihydroxyphenylalanine.

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metabolites in brain tissue, and observations of reduced melanin content in the substantia nigra pars compacta (17, 18). Patients with Rett syndrome served as a control group for the potential effects of anesthesia and reduced caudate volume on the DA transporter function (*vide infra*).

SUBJECTS, MATERIALS, AND METHODS

We studied 6 patients with classical LND (HPRT levels < 1% of normal) (age range, 19–35 years; mean age, 23.7 ± 2.3 (SEM) years), 3 patients with Rett syndrome (age range, 18–23 years; mean age, 20.0 ± 1.5 years), and 10 age-matched normal volunteers (age range, 19–46 years; mean age, 28.8 ± 2.4 years) who were free of psychiatric or neurological disease. None of the LND patients took neuroleptic drugs that bind to DA nerve terminals; two used valium which was discontinued 3 weeks before the study, and one LND patient was taking anticonvulsants (mysoline and phenobarbital). None of the Rett syndrome patients took neuroleptics, but all were previously treated with anticonvulsant drugs. None of the normal control group ever took psychotropic drugs.

The PET studies of the LND and Rett syndrome patients were conducted using light general anesthesia to prevent involuntary movements. Studies in rodents demonstrated that anesthesia does not affect the binding of ligands to the DA transporter (19). The anesthesia procedure consisted of rectal methohexital, inhalation of nitrous oxide, and administration of pancuronium. Subjects were intubated and mechanically ventilated (end tidal $\text{CO}_2 = 40 \pm 5$ mmHg) by an anesthesiologist (M.Y.). Heart rate and blood pressure did not vary >10% from the baseline. The normal controls were not sedated. The specific activity of [^{11}C]WIN-35,428 (<10 μg mass/injection) was greater than 1 $\mu\text{Ci}/\text{pmol}$ (i Ci = 37 GBq). Fifty individual time frames were acquired by a GE 4096+ whole-body tomograph during a 90-min interval (20). HPLC analysis of radiolabeled metabolites in the radial artery plasma samples used the method of Wong *et al.* (20). To allow for reproducible positioning in the PET scanner, an x-ray computed tomography (CT) scan was performed. In five LND patients, the eight normal control subjects, and the three Rett syndrome patients, volumetric magnetic resonance imaging (MRI) images were acquired with a spoiled grass sequence (SPGR), (TE, 5.0 ms; TR, 35 ms; flip angle, 45°; field of view 24 cm) yielding 124 contiguous slices 1.5 mm thick, and 256×192 image matrix. The images were then co-registered to the PET scans using the program REGISTER (21).

The three methods of analysis used to estimate DA transporter binding involved either (i) the caudate- or putamen-to-cerebellum ratio measured during the last three frames of PET imaging (≈ 72 –90 min postinjection) or (ii) the binding potential, which is the ratio of the dopamine transporter density (B_{max}) to the affinity (K_d) and volume of distribution of the tracer ligand in brain relative to water (V_d), in other terms, $B_{\text{max}}/(K_d \cdot V_d)$. This binding potential was estimated by linear or nonlinear regression. These methods have been described previously. Briefly stated, the linear method is based on the graphical approach of estimating rate constants (22, 23) and the nonlinear regression is an estimation of the binding potential with the partition coefficient constrained to be equal in both the striatum and the cerebellum (20). In both cases, the analysis is based upon a three-compartment model consisting of plasma, free and nonspecifically bound tracer ([^{11}C]WIN-35,428) in brain, and tracer bound to the DA transporter. The rate of binding from the plasma to the free/nonspecifically bound compartment is denoted as K_1 , the reverse rate constant is denoted by k_2 ; the forward rate constant from the free/nonspecifically bound compartment to the bound compartment is k_3 and thus represents the binding to the transporter; and the tracer dissociation is denoted by k_4 . In the linear approach, the k_3/k_4 ratio is estimated as a parameter. The

k_3/k_4 ratio is a PET scan quantitative measurement of the ratio of transporter receptor density to the transporter affinity—i.e., the binding potential $B_{\text{max}}/(K_d \cdot V_d)$. The nonlinear approach involves a separate estimation of K_1 and k_2 in the cerebellum and then in the caudate and putamen, thus four separate rate constants are obtained; the ratio of K_1/k_2 is constrained to be equal to that derived in the cerebellum.

The volumes of the basal ganglia were measured from the T1 weighted SPGR MRI scans, which used contiguous slices from just superior to the lateral ventricles through the aqueduct of Sylvius, in an oblique axial projection parallel to the AC-PC line. The lateral ventricles (excluding the temporal horns), the head and body of the caudate nucleus, the putamen, and the globus pallidi were manually outlined by a trained operator. Three-dimensional volumetric images were reconstructed with standard clinical software (ISG, Mississauga, Ontario). Blinded repeat measurements of these structures demonstrate <10% reader variability.

Two-tailed unpaired Student's *t* tests and nonparametric comparisons were employed. The level of significance chosen was $P < 0.05$, after the appropriate correction for multiple comparisons.

RESULTS

PET Studies with [^{11}C]WIN-35,428 in LND, Rett Syndrome, and Normal Controls. In LND, a significant reduction was observed with [^{11}C]WIN-35,428 binding in the caudate (56%) and in the putamen (73%) when compared to both normal control subjects and Rett syndrome patients (Tables 1 and 2). All three methods of estimating DA transporter binding (listed in the tables) showed dramatic reduction in LND compared to healthy control subjects and Rett syndrome patients. The quantification of the binding for each method is summarized in Tables 1 and 2.

The reduced accumulation of WIN-35,428 binding in two patients with LND contrasted with healthy control subjects is illustrated in Fig. 1. The results for all 6 LND subjects, 3 Rett syndrome patients, and 10 control subjects are presented graphically in Fig. 2 for the k_3/k_4 constrained method only.

Because all patients with LND are male, a specific comparison with male controls was carried out and showed significant differences in the measurements of the caudate ($P < 0.03$) and the putamen ($P < 0.008$). When LND patients were contrasted to Rett syndrome patients, the results were also striking for the caudate and putamen ($P < 0.003$). Nonetheless, when Rett syndrome patients were compared to normal controls, there were no statistical differences in [^{11}C]WIN-35,428 binding to the DA transporter in either the caudate or the putamen.

MRI Studies/Partial Volume Correction. In this study five patients with LND underwent volumetric measurements using

Table 1. Quantification of [^{11}C]WIN-35,428 binding in caudate

Group	<i>n</i>	Ratio	Method of analysis	
			Nonlinear, $B_{\text{max}}/(K_d \cdot V_d)$	Linear, $B_{\text{max}}/(K_d \cdot V_d)$
Normal	10	3.24 ± 0.22	6.81 ± 0.57	4.53 ± 0.38
LND	6	$1.63 \pm 0.33^*$	$2.51 \pm 0.30^*$	$2.00 \pm 0.25^*$
RS	3	4.18 ± 0.40	5.35 ± 0.26	4.57 ± 0.31

Values in the table are presented as mean \pm SEM for each method of analysis. Values are not partial volume corrected. For the ratio method, the level of reduction was 49.7% and 61% compared to normal and Rett syndrome (RS) patients, respectively. For the nonlinear analysis, the level of WIN-35,428 binding was reduced by 63% and 53% compared to normal and Rett syndrome patients, respectively. Using linear calculations, the values were reduced by 56% compared to both normal and Rett syndrome patients.

* $P < 0.05$ when compared to normal or Rett syndrome patients.

Table 2. Quantification of WIN-35,428 binding in putamen

Group	n	Ratio	Method of analysis	
			Nonlinear, $B_{max}/(K_d \cdot V_d)$	Linear, $B_{max}/(K_d \cdot V_d)$
Normal	10	3.39 ± 0.29	6.57 ± 0.43	5.42 ± 0.52
LND	6	1.23 ± 0.22*	1.63 ± 0.16*	1.35 ± 0.09*
RS	3	4.37 ± 0.36	4.95 ± 0.72	4.29 ± 0.5

Values in table are presented as mean ± SEM for each analysis. For the ratio method, binding in LND was reduced by 64% and 72% when compared to normal and Rett syndrome (RS) patients, respectively. For the nonlinear calculation, binding in LND was reduced by 75% and 67% when compared to normal and Rett syndrome patients, respectively. For the linear calculation, binding in LND was reduced by 75% and 68% compared to normal and Rett syndrome patients, respectively. *P < 0.05 when compared to Rett syndrome and normal patients.

MRI. MRI was contraindicated in a 6th patient due to the placement of a metal implant. None of the five LND patients showed a significant reduction in putamen volume (LND = 4006 ± 978 mm³ (1 SD); normal = 4171 ± 460 mm³, P < 0.69). However, a significant decrease of 30% in caudate volume (LND = 2644 ± 571 mm³; normal = 3753 ± 561 mm³, P < 0.005) was documented and compared to normal controls. To ensure that the possible reduction in caudate volume would not confound the results of the PET analysis, data from LND subjects were examined by using a rigorous partial volume correction of the time activity curves of the caudate and putamen (24–26). In the caudate, the structure most involved in the volumetric MRI findings, partial volume correction resulted in a greater decrease of the caudate–cerebellum ratio in patients compared to normal control subjects (27). The

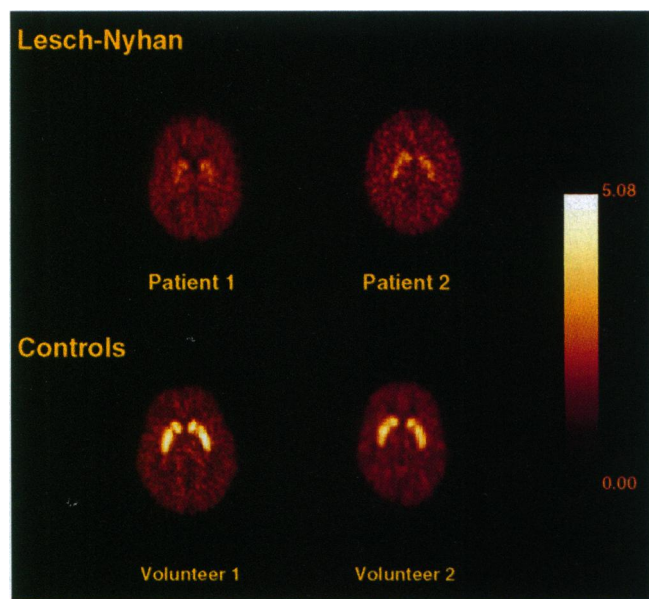


FIG. 1. [¹¹C]WIN-35,428 PET images in two LND and control subjects, ages 22 (Left) and 35 (Right) years. They were acquired and summed between 70 and 90 min postinjection and pass through the caudate and putamen in a transverse plane parallel to the canthal–mental line. They were individually corrected for injected radioactivity, and then the image containing the caudate and putamen was divided by the cerebellum on a pixel-by-pixel basis. The images were scaled to the global highest and lowest values for all of the images of all subjects, and hence were scaled to a common maximum and minimum. The control subjects are matched for age. These images demonstrate dramatic reduction in DA transporter binding in the basal ganglia, which is consistent with a reduction in the number of DA terminals.

results of one example are shown in Fig. 3. The partial volume corrected measurements show that the reduction in the pre-synaptic transporter binding in LND is not due to differences in caudate volume.

DISCUSSION

The DA transporter is an important element of the DA-containing neuron and is uniquely localized to dopaminergic nerve terminals in the brain. The DA transporter has been characterized, cloned, and subjected to structure-function studies (28–31). As noted in the introductory comments, destruction of dopaminergic neurons with neurotoxins results in a major reduction of markers such as WIN-35,428 in brain regions with dopaminergic innervation.

To our knowledge, the finding of low DA transporter binding with WIN-35,428 in LND provides the first *in vivo* documentation of a dopaminergic reduction in this disease. The reduction in DA could be due to a loss of DA neurons or DA nerve terminals. However, the works of Lloyd *et al.* (3) in humans and Jinnah *et al.* (6) in mutant mice are consistent with DA terminal loss; direct examination of the substantia nigra would be necessary to determine if there is definite neuronal loss. Our findings of a DA reduction is consistent with (i) the reported decreased content of DA and its synthesizing en-

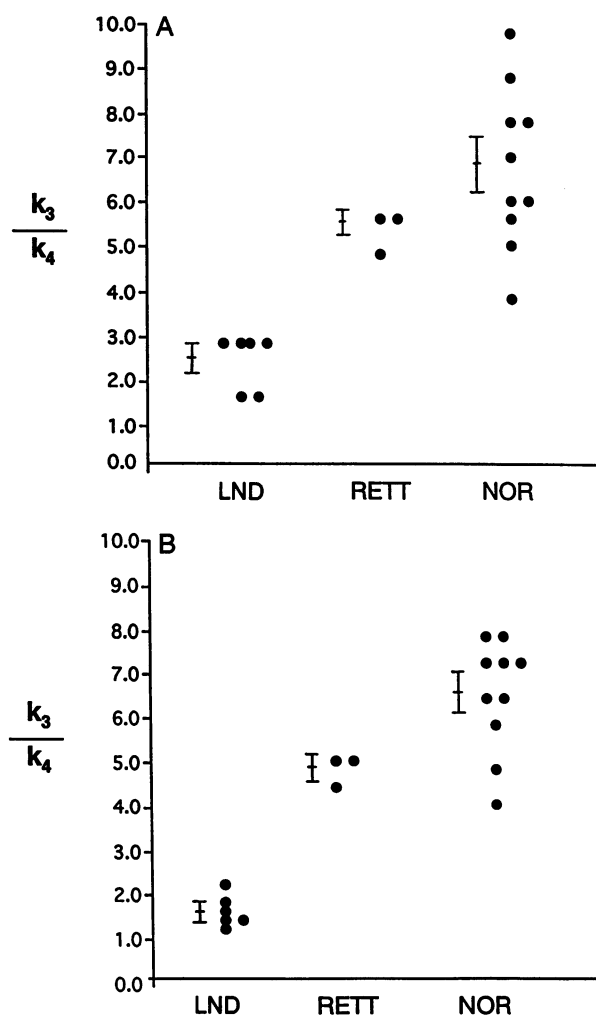


FIG. 2. Scattergrams depicting [¹¹C]WIN-35,428 binding for LND, normal control subjects, and Rett syndrome patients in the caudate (a) and putamen (b) by using the k_3/k_4 constrained method. These scattergrams clearly show the absence of overlap of the LND cases with Rett syndrome or normal control subjects. Bars show mean ± SEM.

zymes in the caudate (65%) and putamen (90%) in postmortem brain tissue of three subjects (3), (ii) the reduced levels of DA metabolites found in the spinal fluid of LND patients (33, 34), and (iii) the reduction in DA and its synthesizing enzymes in an HPRT-deficient mouse mutant (6). Consequently, the loss of DA accompanying a loss of HPRT activity is now established with the addition of the present *in vivo* data in living LND patients. Furthermore, behavioral findings from rats with self-injurious behavior whose dopaminergic neurons were lesioned with 6-hydroxydopamine at 3 days of age suggests that reduced DA contributes to this unique behavior in LND (4). Although different in character from Parkinson disease, our findings are consistent with the movement disorder present in LND (e.g., dystonia and choreiform movements). These results are also consistent with findings which

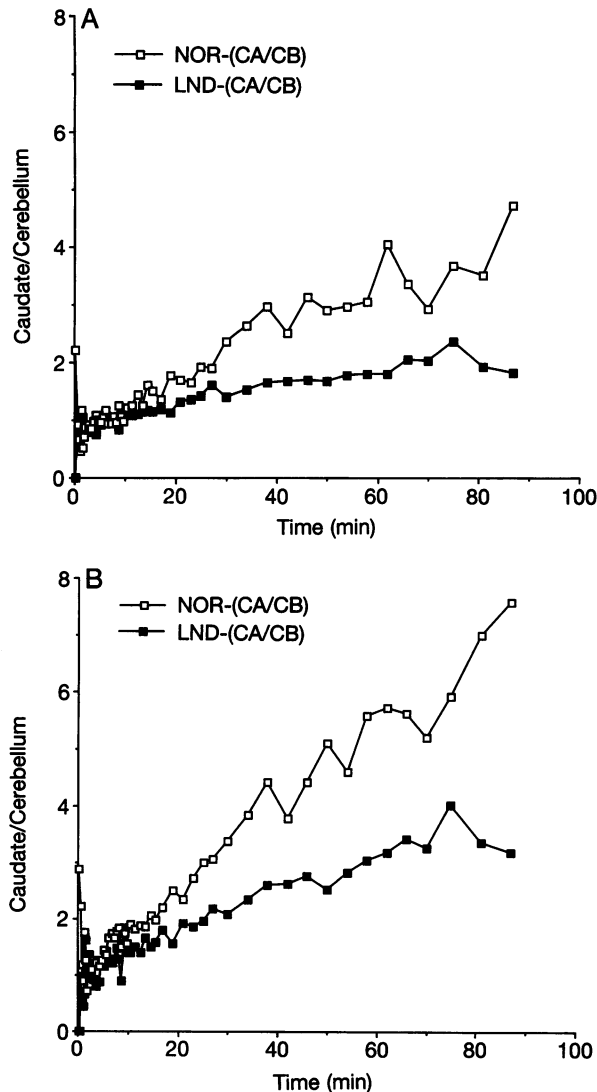


FIG. 3. Effects of partial volume correction (on caudate quantification) in a patient with LND. Time activity curves comparing a single LND patient to a normal control for uncorrected WIN-35,428 binding (a) and with partial volume correction (b). Partial volume correction in this example increases the normal and patient DA transporter binding (using the method of constrained k_3/k_4) from 3.86 to 10.4 and from 1.55 to 2.50, respectively. In this single example, the original quantification demonstrated a 60% reduction in the LND patient, but partial volume correction revealed a reduction of 76%. We conclude that reductions in the caudate structures do not in fact confound the finding, and that there is a dramatic reduction in DA transporter binding in the caudate and putamen of LND patients compared to controls and Rett syndrome patients.

state that disturbances of components of corticostriate circuits underlie hypokinetic and hyperkinetic disorders (35–37). Additional research is needed to identify the precise functional neuropathology of LND in the DA-containing brain regions.

The LND PET studies were carried out with anesthetic agents, thus raising the issue of whether or not anesthetics affect binding to the DA transporter. However, no reduction of transporter density was found in animal studies that used the same anesthetic agents (data not shown) (19). Also, the Rett syndrome PET studies used the same anesthesia protocol. The lack of a significant DA transporter reduction in Rett syndrome suggests that anesthesia, in itself, does not result in the reduced WIN-35,428 binding observed in LND.

In experiments that depend on binding as an index of neuronal density, one potential difficulty that could lead to an erroneous interpretation of the findings is the up- or down-regulation of transporter sites occurring in response to the changing synaptic levels of DA (32, 38). From the PET measurement alone, it is not possible to determine whether the decreased WIN-35,428 binding *in vivo* is due to a down-regulation of the DA transporter. Both down-regulation and loss of DA-containing neurons reduce the number of DA transporter sites. However, findings from the HPRT-deficient mice (6) and from LND patients support the conclusion that a significant loss of dopaminergic nerve terminals and/or neurons occurs (3). Furthermore, a reduction of the DA transporter in the presence of normal DA content would probably result in normal or elevated DA metabolites in cerebral spinal fluid and not in the reduced level of DA metabolites found in LND (33, 34).

Another limitation in DA transporter analysis that must be considered is the difference in volumes of the caudate and the putamen in patients compared to controls. Although we did find some evidence of reduced caudate volume in the LND cases, partial volume corrections of the volumes resulted in an even greater reduction of the DA transporter in LND (Fig. 3). In Rett syndrome, using MRI, Reiss *et al.* (39) have documented a 30% reduction in caudate volume. Since patients with LND had similar reductions, Rett syndrome patients serve as a control group to compare caudate size with the LND group.

The present findings might be refined by estimating absolute transporter densities (B_{max}) instead of using the ratio of density to affinity. Other methods, using receptor blocking agents such as mazindol to partially occupy DA transporter sites, have been proposed as a means for absolute quantification (20) (D.F.W., unpublished observations). However, the methods of kinetic analysis employed in this study currently provide the best available estimates of DA transporter density and affinity. Regardless of any uncertainties about the absolute degree, the evidence for the reduction of DA transporters in LND patients remains striking and demonstrates that current *in vivo* methods for measuring the binding potential of the DA transporter quite adequately detect an *in vivo* decrease in DA content in LND.

Confirmation by our *in vivo* PET studies of a dopaminergic abnormality in LND lends support to the hypothesis that the neonatal reduction of DA in LND leads to different motor symptoms in this disorder than those observed in Parkinson disease (4, 5). Developmental abnormalities in striatal organization may account for the motor symptoms in LND. DA reduction may also be linked to self-injury. The self-injury associated with LND worsens when LND patients are given L-dopa (34). In addition to these clinical observations, the hypothesis concerning a neonatal reduction of DA has received particular support from animal studies that compared the consequences of reducing DA neonatally in rats and assessing for self-injury when the rats became adults. As seen in LND patients, rats lesioned as neonates and given L-dopa

showed an increased incidence of self-injury, whereas rats lesioned as adults showed no self-injury. Therefore, the identification of a drug treatment that could block the self-injury caused by an L-dopa challenge in the neonatal-6-hydroxydopamine model is actively underway. Additionally, the development of a therapy to prevent this DA deficiency in LND during the developmental period is also a goal of future investigations. The availability of the HPRT-deficient mouse with a DA reduction in the brain will provide a model to carry out these latter studies (6).

In summary, the findings demonstrate that the DA transporter can be imaged *in vivo* in developmental disorders with PET imaging. The data collected in LND clearly illustrates the value of PET imaging in a neurodevelopmental disorder. It documents the reduction in binding of a ligand to the DA transporter and definitively indicates that DA content is reduced in the neurodevelopmental disorder, LND.

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