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Age-dependent Motor Deficits and Dopaminergic Dysfunction in DJ-1 Null Mice*

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Mutations in the DJ-1 gene were recently identified in an autosomal recessive form of early-onset familial Parkinson disease. Structural biology, biochemistry, and cell biology studies have suggested potential functions of DJ-1 in oxidative stress, protein folding, and degradation pathways. However, animal models are needed to determine whether and how loss of DJ-1 function leads to Parkinson disease. We have generated DJ-1 null mice with a mutation that resembles the large deletion mutation reported in patients. Our behavioral analyses indicated that DJ-1 deficiency led to age-dependent and task-dependent motoric behavioral deficits that are detectable by 5 months of age. Unbiased stereological studies did not find obvious dopamine neuron loss in 6-month- and 11-month-old mice. Neurochemical examination revealed significant changes in striatal dopaminergic function consisting of increased dopamine reuptake rates and elevated tissue dopamine content. These data represent the *in vivo* evidence that loss of DJ-1 function alters nigrostriatal dopaminergic function and produces motor deficits.

Mutations in DJ-1 were recently identified in an autosomal recessive form of early-onset familial Parkinson disease (PD)[1] (1). The first reported mutation involves one large deletion of the first 5 exons and part of the promoter and another mutation was a missense mutation (L166P) that might cause instability of the DJ-1 protein by preventing it from folding properly and forming homodimers (2–5). Since this first report, a number of other mutations of DJ-1 including deletion mutations, point mutations, and a frameshift mutation have been found to cause PD (6–10). These studies suggest that the loss of the normal function of DJ-1 leads to PD.

However, the nature of the normal function of DJ-1 and the mechanism by which DJ-1 deficiency leads to PD are not well established. Studies prior to the report of its association with PD suggested that DJ-1 might play a role in oncogenesis (11), male fertility (12, 13), control of protein-RNA interaction (14), and in modulating androgen receptor transcription activity (15, 16). In addition, the DJ-1 protein was shown to be responsive to oxidation (17, 18), suggesting a potential role in oxidative stress, a process often implicated in PD. Studies on PD-linked DJ-1 mutations indicate that wild-type, but not mutant, DJ-1 protects cells from oxidative stress (19–21). Canet-Aviles *et al.* (22) reported that oxidation of the Cys^{106} residue in DJ-1 could lead to its relocalization in mitochondria and protect cells from mitochondrial damage. Structurally, DJ-1 closely resembles the members of the ThiJ/PfpI family that have protease and chaperone activities (23–27). Recent biochemical studies suggested that DJ-1 might have protease (5) and redox-dependent chaperone activities (28). Therefore, putative functions of DJ-1 seem to converge on the common pathogenesis of PD implicated in other genetic and sporadic forms of PD.

Despite those new insights into the biochemical and cellular functions of DJ-1, the *in vivo* evidence of how the loss of DJ-1 function leads to PD has not been presented. Specifically, it will be important to find out whether animal models that harbor similar mutations to those found in PD patients will develop dopamine neuron degeneration and/or parkinsonian motor symptoms. Since human genetic studies suggest that a loss-of-function of DJ-1 is responsible for the pathogenesis of this genetic form of PD, we have generated DJ-1 null mice with a mutation that resembles the large deletion mutation reported in patients. Our behavioral analyses indicate that DJ-1 deficiency leads to age-dependent motoric behavioral deficits. Even though no obvious dopamine neuron loss was found in 6-month- and 11-month-old mice, neurochemical examination of dopaminergic function revealed significant alterations in tissue dopamine content and reuptake in DJ-1 null mice.

MATERIALS AND METHODS

Generation of DJ-1 Null Mice—We generated DJ-1 null mice by deleting 9.3-kb genomic DNA including the first 5 exons and part of the promoter region of DJ-1 gene to mimic the deletion mutation in humans (1) (Fig. 1*A*). Both the 5.4- and 3.3-kb fragments of the targeting construct were amplified by PCR using genomic DNA isolated from E14Tg2A.4 embryonic stem cells (BayGenomics) as a template. The selection marker PGK-neo-poly(A) was flanked by FRT sequences. E14Tg2A.4 embryonic stem cells were electroporated (800 V and 3 microfarads) with 30 μg of linearized targeting construct. G418-resistant clones were screened by Southern blot for homologous recombination with a 5′-external probe. Positive cells were injected into C57BL6/J blastocysts to generate chimeras, which were then mated with C57BL6/J wild-type mice to generate heterozygotes. Heterozygous mutant mice on a 129 × C57B/6 mixed background were bred to generate DJ-1 null mice and their wild-type littermate controls for experiments.

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[1] The abbreviations used are: PD, Parkinson disease; TH, tyrosine hydroxylase; SNc, substantial nigra pars compacta; THir, TH-immunoreactive; DAT, dopamine transporter; VMAT2, vesicular monoamine transporter-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPLC, high performance liquid chromatography.

Mice were genotyped by multiplex PCR on genomic DNA extracted from tail snips (Fig. 1*B*): first primer pair amplifies part of intron 6 of DJ-1 (present in all mice); second primer pair amplifies part of *neo*^{r} (absent in wild-type mice); third primer pair amplifies part of intron 3 (absent in homozygous mutants). The genotypes of some mice were confirmed by Southern blot analysis (Fig. 1*C*). The absence of DJ-1 expression was confirmed by *in situ* hybridization. A full-length mouse DJ-1 cDNA was amplified by reverse transcription-PCR and used as an *in situ* hybridization probe. All animal procedures were approved by the Institutional Animal Care and Usage Committee of The University of Chicago.

Behavioral Studies—All mice were kept on a 06:00–18:00 light cycle with *ad libitum* food and water. Behavioral tests were performed during the light period.

Open Field Test—Each mouse was placed in an open field chamber (40 cm long × 40 cm wide × 37 cm high, Med Associates). Illumination of open field was set to 20 lux. No background noise was provided. They were monitored by infrared beams that record the location and path of the animal (locomotor activity) as well as the number of rearing movements (vertical activity). Data were collected in 5-min trials for six trials, and the average was reported.

Rotarod Test—Mice were first trained to stay on the rod of the rotarod (Columbus Instrument) at a constant speed of 5 rpm for at least 1 min. Following training, mice were tested for a total of three trials with an accelerating speed of 0.2 rpm/s, starting at 5 rpm. The latency to fall was recorded for each trial, and the average of three trials was reported.

Adhesive Tape Removal Task—Adhesive tapes (Avery labels) of five sizes (0.625, 0.5, 0.375, 0.25, 0.125 in^2) were placed on the forehead in the above order. To remove the tape, mice typically raised both forepaws and swiped off the tape. Each trial was given a score equal to the size of the largest tape the mouse was unable to remove within 60 s (5 to 1, a higher score indicates worse performance). Results were obtained from the average over two trials.

Tyrosine Hydroxylase (TH) Immunohistochemistry and Stereology—TH catalyzes the rate-limiting step of dopamine synthesis and was used as a marker for dopamine neurons in the substantial nigra pars compacta (SNc). The number of TH-immunoreactive (THir) cells was assessed using an unbiased stereological procedure (29). Briefly, mice were perfused by 4% paraformaldehyde, post-fixed in 4% paraformaldehyde, and cryoprotected in 30% sucrose. The entire brain was sliced on a sliding microtome into consecutive 40-μm sections. Every third section was immunohistochemically processed for TH and NeuN. For TH staining, rabbit anti-mouse TH antibody (1:1000, Pel-Freez), biotinylated horse anti-rabbit IgG (1:500; Vector Laboratories), and peroxidase-conjugated avidin-biotin complex (VECTASTAIN Elite ABC kit, Vector Laboratories) were used. The reaction was visualized by using Sigmafast diaminobenzidine tablets (Sigma) with the addition of nickel sulfate. For NeuN staining, mouse anti-mouse NeuN antibody (1:1000, Chemicon), biotinylated goat anti-mouse IgG (1:500; Vector Laboratories) and diaminobenzidine without nickel sulfate were used. The estimation of the total number of THir neurons in the SNc was determined using the computerized optical fractionator probe (Stereo Investigator 6 from MicroBrightField), which allowed for the stereological estimation of THir cells in the entire structure independent of size, shape, orientation, tissue shrinkage, or anatomical level. The 100 × 100-μm grid and 40 × 40-μm counting frame were used. Sections were counted in rostral-caudal order. The most rostral section containing THir neurons was designated as the first section. The absolute THir neuron number was directly calculated without estimating reference volume. The Gundersun's coefficient of error was 0.06 (m = 1) in this study.

Immunofluorescence Double Labeling—Rabbit anti-mouse TH (1:500, Pel-Freez) and mouse anti-mouse α-synuclein (1:500, BD Transduction Laboratories) antibodies were used to detect α-synuclein expression in dopamine neurons. Mouse anti-mouse TH (1:500, BD Transduction Laboratories) and rabbit anti-ubiquitin antibodies (1:500, Dako) were used to detect protein ubiquitination in dopamine neurons. All fluorescence secondary antibodies were purchased from Jackson ImmunoResearch Laboratories and used according to the manufacturer's instructions. Sections were analyzed under a Zeiss Confocal LSM510 Microscope.

HPLC Assessment of Brain Tissue Content of Dopamine and Metabolites—Mice were killed by cervical dislocation. Dissected striata of adult mice were homogenized in 0.1 M perchloric acid containing 1 × 10^{-6} M methyldopa as an internal standard. Homogenates were centrifuged for 10 min at 1500 × *g*. Supernatants were filtered through an YM-10 Microcon centrifugal filter unit (Millipore) and analyzed for dopamine and metabolites using high performance liquid chromatography with electrochemical detection. Dopamine and metabolites were separated on a reverse-phase column (Velosep RP-18, 3 μm, 100 × 3.2 mm, PerkinElmer Life Sciences) with a mobile phase consisting of 0.1 M phosphate buffer with 0.6 mM octyl sodium sulfate, 0.1 mM EDTA, and 3% methanol (pH 2.6) at a flow rate of 0.8 ml/min. Samples (25 μl) were injected with a Rheodyne injector, and the compounds were analyzed using a coulometric detector with analytical cell (model 5014, ESA). The potential for the first and second cell was set at −100 and +310 mV, respectively. A guard cell (model 5020, ESA, potential +330 mV) was placed before the injector. Levels of dopamine and metabolites detected in the supernatants were calculated using internal standards. Final concentrations of dopamine and metabolites were expressed per protein amount. Protein levels were measured by BCA protein assay kit (Pierce).

Western Blot—Mice were killed by cervical dislocation. Brains were dissected and frozen immediately on powdered dry ice and stored in −80 °C until use. Frozen brains were lysed in NTE buffer (150 mM NaCl, 50 mM Tris pH 8.0, 5 mM EDTA, 0.25 mM phenylmethylsulfonyl fluoride, 1% protease inhibitor mixture (Sigma), 2% β-mercaptoethanol, 2% SDS), and 50 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis. Proteins were electrophorectically transferred to PVDF membrane and nonspecific sites blocked in 5% nonfat dry milk in TBS (135 mM NaCl, 2.5 mM KCl, 50 mM Tris, 0.1% Tween 20 (pH 7.4)). Membranes were then incubated with various primary antibodies in TBS with 2% nonfat dry milk: rat anti-mouse dopamine transporter (DAT) antibody (1:4000, Chemicon), rabbit anti-mouse TH antibody (1:2000, Pel-Freez), goat anti-mouse vesicular monoamine transporter-2 (VMAT2) antibody (1:500, Santa Cruz Biotechnology), mouse anti-mouse α-synuclein antibody (1:2000, BD Transduction Laboratories), rabbit anti-mouse androgen receptor antibody (1:1000, Abcam), mouse anti-mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:200,000, Abcam). Signals were then detected by horseradish peroxidase-conjugated secondary antibodies (ICN) and enhanced chemiluminescence (Pierce). Membranes were then stripped and reprobed with the monoclonal mouse antibodies against mouse β-actin (1:200,000, Abcam) to confirm equal loading of samples.

Protein Carbonyl Analysis—The commonly used indicator of protein oxidation is protein carbonyl content (30). The OxyBlot protein oxidation detection kit from Chemicon was used following the manufacturer's instructions.

Cyclic Voltammetry in Brain Slices—Mice were killed by decapitation, and the brain was rapidly removed and placed in ice-cold, preoxygenated (95% O_2/5% CO_2) modified Kreb's buffer. The tissue was then sectioned into 400-μm-thick coronal slices containing the caudate-putamen and nucleus accumbens with a vibrating tissue slicer (Leica VT100S, Leica Instruments). Slices were kept in a reservoir of oxygenated Krebs buffer at room temperature until required. Thirty minutes before each experiment, a brain slice was transferred to a submersion recording chamber. The slices were perfused at 1 ml/min with room temperature (20 °C) oxygenated Krebs and allowed to equilibrate. The Kreb's buffer contained (in mM): NaCl, 126; KCl, 2.5; NaH_2PO4, 1.2; $CaCl_2$, 2.4; MgCl, 1.2; $NaHCO_3$, 25; glucose, 11; HEPES, 20; and L-ascoribic acid, 0.4 (pH 7.4). Dopamine release was evoked by a single, rectangular, electrical pulse (300 μA, 2 ms per phase, biphasic) applied every 5 min. Dopamine was detected by using fast-scan cyclic voltammetry as described earlier (31, 32). Electrodes were placed in the dorsal and ventral portion of the caudate-putamen and in the core and shell of the nucleus accumbens.

Data Analysis—Data were analyzed using StatView 5.0.1. Unpaired two-tailed Student's *t* test was used when genotype was the only grouping variable. Analysis of variance was used when genotype was not the only grouping variable and when data were collected in a single trial. Repeated measure analysis of variance was used when data were collected in multiple trials.

RESULTS

Generation of DJ-1 Null Mice—DJ-1 null mice were generated by deleting the first 5 exons and part of the promoter to mimic the deletion mutation reported in human PD patients (1) (Fig. 1*A*). The lack of DJ-1 transcript expression in homozygous mutants was confirmed both by reverse transcription-PCR in cultured adult skin fibroblasts (Fig. 1*D*) and by *in situ* hybridization on brain sections (Fig. 1*E*). DJ-1 mRNA signal was detected throughout the wild-type mouse brain by *in situ* hybridization. The high expression level of DJ-mRNA was found in the SNc, cerebellum, cerebral cortex (Fig. 1*E*), hippocampus,

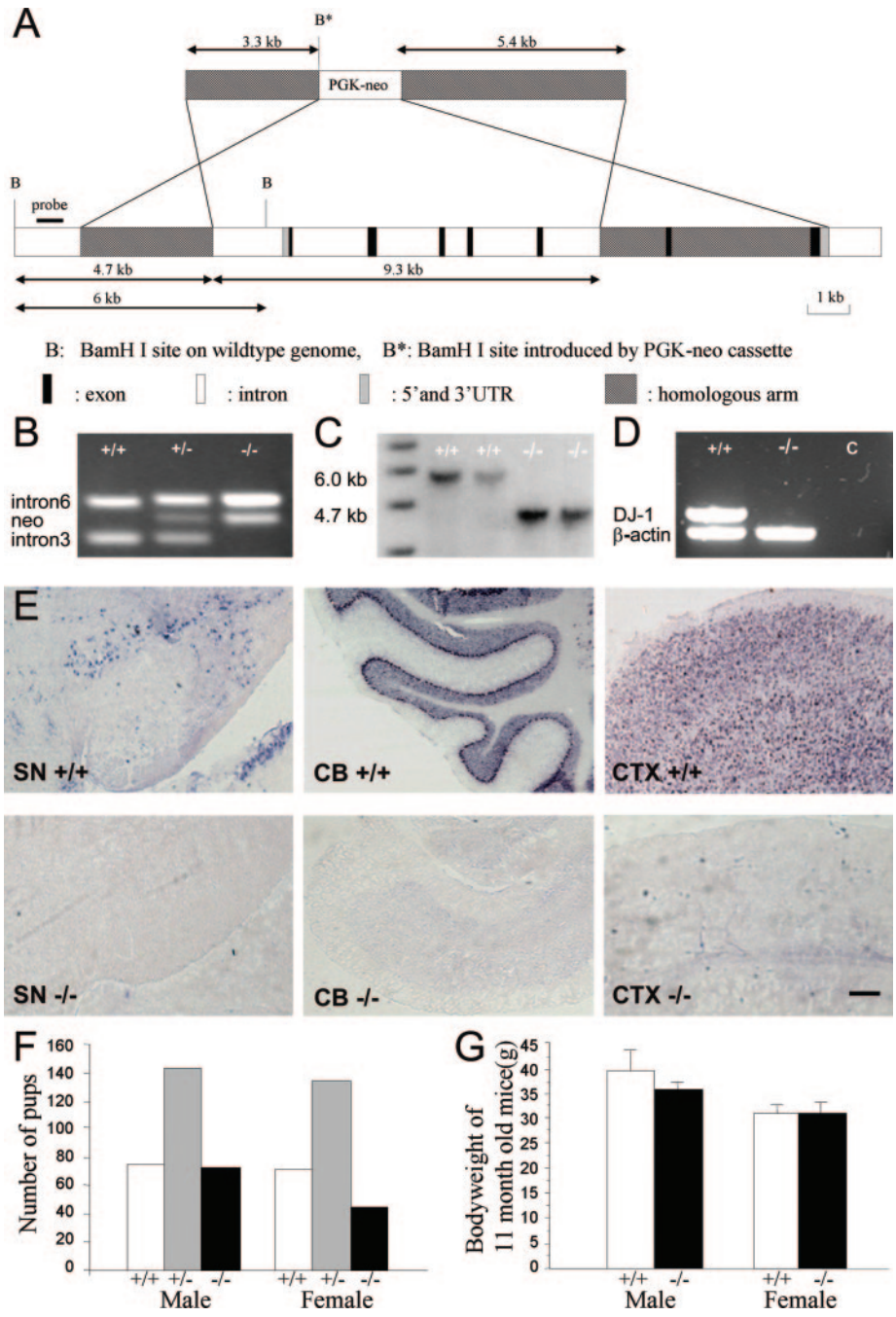

FIG. 1. **Generation of *DJ-1* null mice.** DJ-1 null mice were generated by gene targeting that resulted in 9.3-kb deletion that included the first 5 exons and part of the promoter. *A*, design of the targeting construct. The FRT sequence-flanked selection cassette (FRT-PGK-neo^r-poly(A)-FRT) was inserted in between two homologous arms. BamHI digestion will give a 6-kb band for the wild-type allele and a 4.7-kb band for the mutant allele in Southern blot. *B* and *C*, PCR (*B*) and Southern blot (*C*) analyses of genomic DNA from tail snips confirm transmission of the mutation. *D*, reverse transcription-PCR analysis of mRNA from cultured skin fibroblast confirms the loss of DJ-1 transcript. *E*, *in situ* hybridization demonstrates the loss of DJ-1 transcript in brains of mutant mice. *F*, genotype distribution conforms to Mendelian ratio. The χ^2 test did not reveal significant deviation from Mendelian law ($p = 0.09$). *G*, no significant body weight difference was found among different genotypes in 11-month-old mice ($p = 0.41$; $n = 12$ for each genotype). *Scale bar* is 100 μm. *SN*, substantia nigra; *CB*, cerebellum; *CTX*, cerebral cortex; *UTR*, untranslated region.

striatum, and olfactory bulb (data not shown). Most of the expression was in cells exhibiting neuronal morphology. However, strong expression was also present in the choroid plexus (data not shown). These data are similar to the expression patterns reported by others (33, 34). The ratios of wild-type: heterozygotes:homozygotes of both sexes were consistent with Mendelian inheritance (Fig. 1*F*, $p = 0.09$ by χ^2 test). DJ-1 null mice were viable and appeared indistinguishable from the wild-type or heterozygotes littermates in gross inspection. No significant body weight difference was found between genotypes ($p = 0.41$; Fig. 1*G*).

Age-dependent Motor Deficits in DJ-1 Null Mice—Three independent age groups of DJ-1 null mice (5, 9, and 11 months) and their wild-type littermate controls were subjected to systematic behavioral assessment of various motor functions. In the open field test, DJ-1 null mice displayed decreased locomotor ($p = 0.001$) and rearing ($p = 0.005$) activities (Fig. 2). Such a deficit seemed to be age-dependent (genotype × age interaction: $p = 0.032$ for rearing and $p = 0.068$ for locomotion). *Post hoc* test revealed that only 11-month-old DJ-1 null mice showed significantly impaired locomotion and rearing compared with their wild-type controls ($p = 0.002$ for locomotion and $p = 0.037$ for rearing). There was no significant genotype × sex interaction.

To determine whether more sensitive measures of nigrostriatal dysfunction could differentiate DJ-1 null mice from their wild-type controls at an earlier age, an adhesive tape removal test that has been shown to be a good discriminator in mouse models of PD was employed (35, 36). Behavioral deficits in the adhesive tape remove test could be seen as early as 5 months of age in DJ-1 null mice compared with their wild-type controls (Fig. 3). The behavioral deficit in DJ-1 null mice was more pronounced at 11 months of age than at 5 months ($p = 0.0001$ for overall genotype effect, $p = 0.013$ for 5-month group, and $p = 0.007$ for 11-month group), although there was no statis-

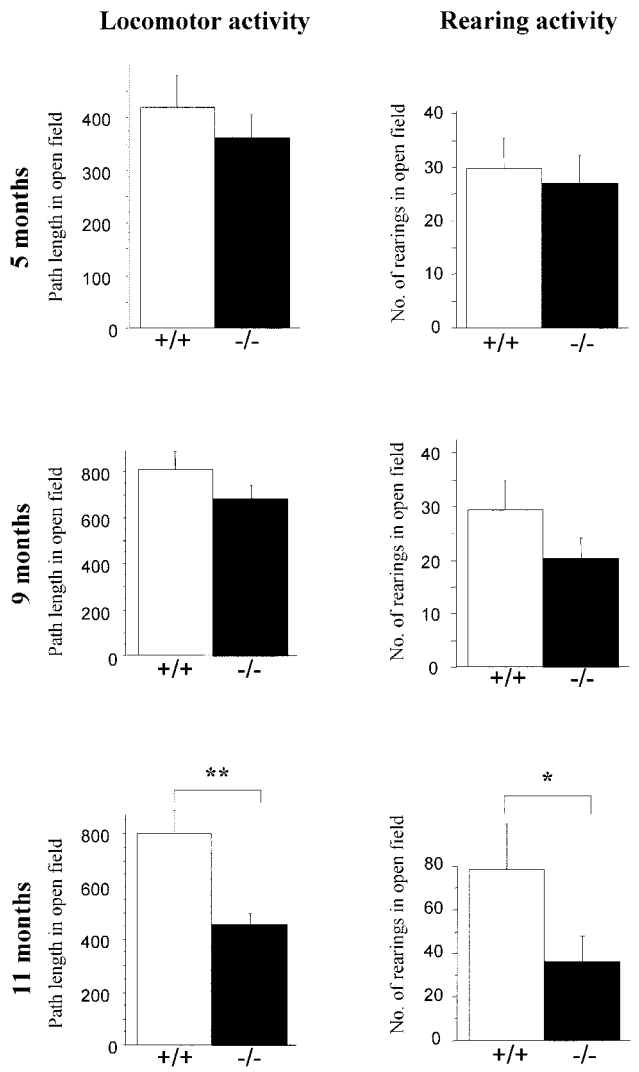

FIG. 2. **Age-dependent motor deficits in DJ-1 null mice.** DJ-1 null mice displayed decreased locomotor ($p = 0.001$) and rearing ($p = 0.005$) activities in the open field. Such a deficit seemed to be age-dependent (genotype × age interaction: $p = 0.032$ for rearing and $p = 0.068$ for locomotion). *Post hoc* test revealed that only 11-month-old DJ-1 null mice were significantly impaired in their locomotor ($p = 0.002$) and rearing ($p = 0.037$). (Number of mice used for each genotype: 16 for 5 months old; 15 for 9 months old; 12 for 11 months old).

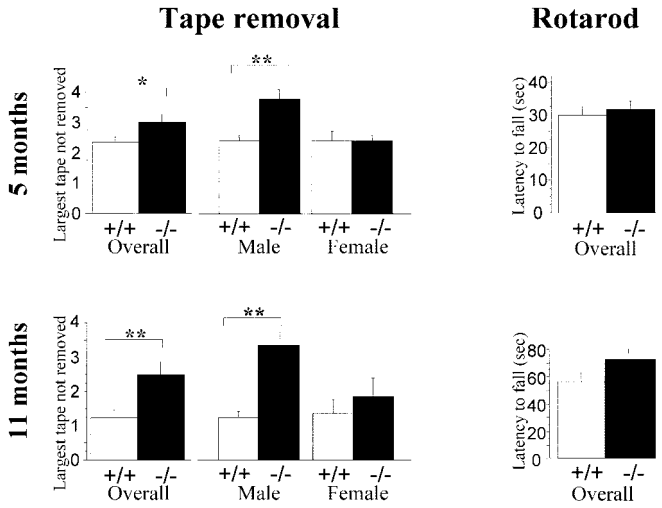

FIG. 3. **Motor deficits in DJ-1 null mice are task-dependent.** The adhesive tape removal test was a more sensitive test, which revealed behavioral deficit in DJ-1 null mice as early as 5 months of age ($p = 0.013$ for 5-month-old mice and $p = 0.007$ for 11-month-old mice). Male mice seemed to be more affected than female mice ($p = 0.0027$ for genotype × sex interaction). *Post hoc* test revealed that only male mice displayed significant deficit ($p < 0.0001$). Rotarod treadmill behavioral test did not detect any genotype differences in any age groups. (Number of mice used for each genotype and sex: 8 for 5 months old; 6 for 11 months old).

tically significant genotype × age interaction ($p = 0.19$). Interestingly, male mice seemed to be more affected than female mice on this task ($p = 0.0027$ for genotype × sex interaction). *Post hoc* tests revealed that only male mice displayed significant deficits ($p < 0.0001$ for males and $p = 0.50$ for females). The rotarod treadmill behavioral test is also widely used as a measure of various neurological dysfunctions. However, the rotarod treadmill test did not detect any genotype differences in any age groups including 11 months of age (Fig. 3).

Functional Alterations in Dopaminergic Neurotransmission in DJ-1 Null Mice—Since functional alterations in dopaminergic neurotransmission may precede obvious dopaminergic neuronal loss or pathological abnormalities, we examined dopamine release, uptake kinetics, and total striatal tissue content of dopamine in DJ-1 null mice and their wild-type controls.

Fast-scan cyclic voltammetry was employed to evaluate electrically stimulated dopamine release and uptake kinetics of the dopamine transporter. The caudate-putamen was divided into dorsal and ventral halves for data analysis. At 4 months of age, DJ-1 null mice had increased stimulated dopamine release (Fig. 4*A*; $p = 0.0002$) and faster uptake (V_{max}) (Fig. 4*E*; $p = 0.0001$) compared with wild-type littermate controls in the dorsal caudate-putamen. However, in ventral caudate-putamen, dopamine release (Fig. 4*B*; $p = 0.61$) and V_{max} for uptake (Fig. 4*F*; $p = 0.18$) were not significantly affected by genotype. There were no significant sex differences.

We further characterized dopamine terminal regions in the core and shell of the nucleus accumbens of DJ-1 null mice. There were no significant differences in dopamine release (Fig. 4*D*; $p = 0.79$) or V_{max} for uptake (Fig. 4*H*; $p = 0.91$) in the nucleus accumbens shell. There was also no significant difference in dopamine release (Fig. 4*C*; $p = 0.47$) in the nucleus accumbens core. However, in the core, there was a trend toward an increased V_{max} value for dopamine uptake (Fig. 4*G*; $p = 0.084$).

To investigate whether the increases in uptake and release reflect changes in dopamine tissue content, the caudate-putamens of 6-month- and 11-month-old mice were dissected out and subjected to HPLC analysis for dopamine and metabolite content. Dopamine levels in DJ-1 null mice were higher than those in wild-type control mice in both age groups ($p = 0.0014$ for genotype effect; $p < 0.0001$ for age effect Fig. 4*I*). There was no significant age × genotype interaction or sex differences. There was an insignificant trend for elevation of 3,4-dihydroxyphenylacetic acid ($p = 0.11$) and homovanillic acid ($p = 0.14$) in DJ-1 null mice compared with wild-type control mice.

We investigated whether the increases in tissue dopamine content, release, and uptake are due to changes in expression levels of dopamine synthesis enzymes or transporters. However, no significant differences between genotypes were seen in TH, DAT, or VMAT2 protein levels in 5-month- (not shown) and 11-month-old (Fig. 4*J*) mouse brains measured by Western blot analysis.

DJ-1 Null Mice Did Not Show Loss of SN Dopamine Neurons or Obvious Neuropathology—To determine whether the behavioral deficits resulted from the loss of dopaminergic neurons, stereological analysis of THir neurons in the SNc was performed in 6-month- and 11-month-old mice. However, comparable numbers of dopaminergic neurons were present in DJ-1 null mice and their wild-type controls (Fig. 5).

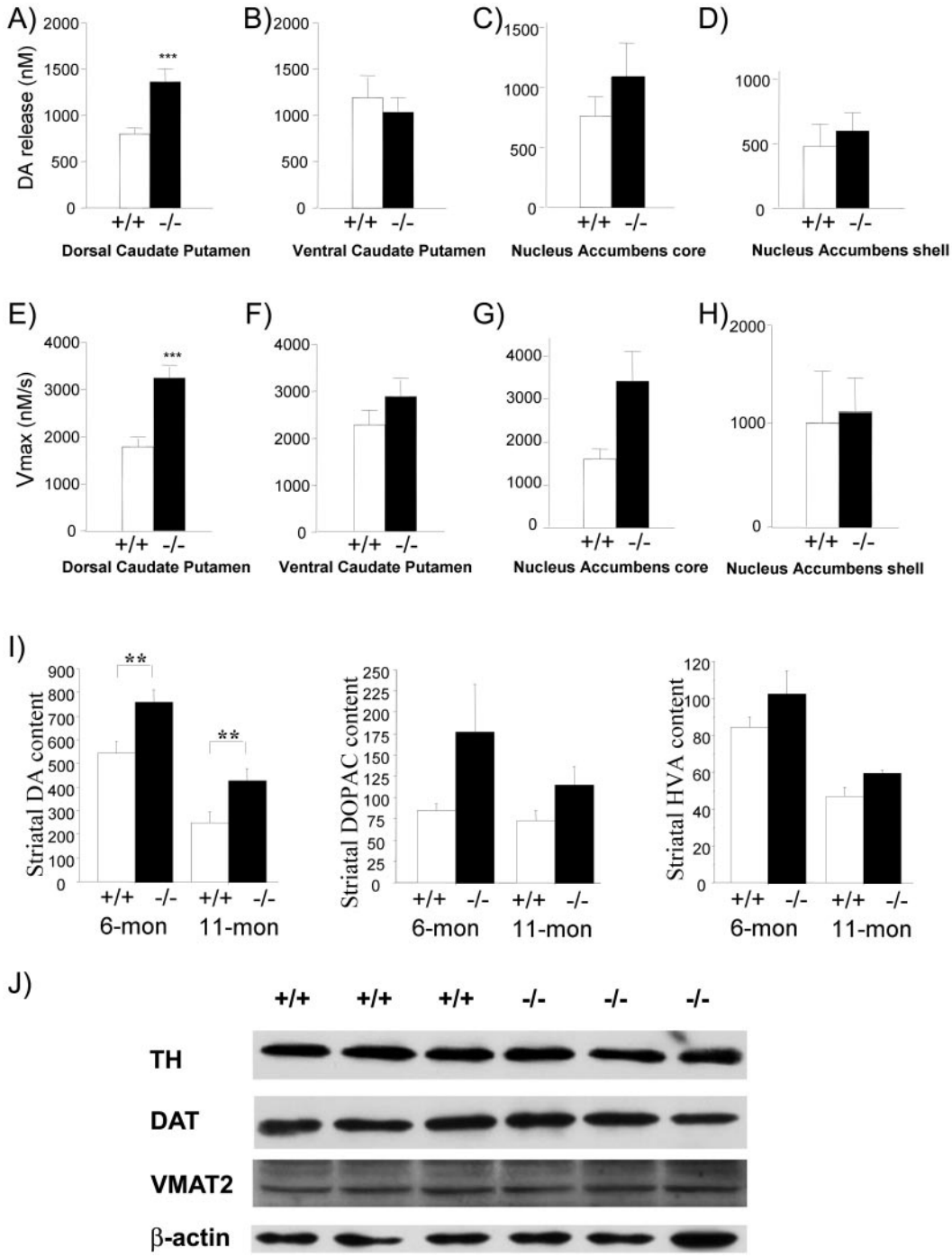

FIG. 4. **Impaired nigrostriatal dopaminergic function in DJ-1 null mice.** *A–H*, cyclic voltammetry examination of dopamine (*DA*) release in response to a single stimulus pulse and maximal uptake rate (V_{max}) in brain slices from 4-month-old mice ($n = 8$ for each genotype). DJ-1 null mice released more dopamine in the dorsal (*A*; $p = 0.0002$) but not in the ventral caudate-putamen (*B*; $p = 0.61$), the nucleus accumbens shell (*D*; $p = 0.79$), or core (*C*; $p = 0.47$). In addition, DJ-1 null mice displayed higher maximal dopamine uptake rate in the dorsal (*E*; $p = 0.0001$) but not in the ventral caudate-putamen (*F*; $p = 0.18$) or the nucleus accumbens shell (*H*; $p = 0.91$). There was a trend that DJ-1 null mice had higher maximal dopamine uptake rate in the nucleus accumbens core than wild-type mice (*G*; $p = 0.084$). *I*, DJ-1 null mice had elevated dopamine (*DA*) (ng/mg of protein) levels in the striatum than wild type mice ($p = 0.0014$; $n = 13$ for each genotype of 6-month-old and $n = 7$ for each genotype of 11-month-old group). There was a trend for higher 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (*HVA*) levels in DJ-1 null mice than in than wild type mice. *J*, Western blot analysis of TH, DAT, and VMAT2 levels in 11-month-old brains did not reveal any genotype differences.

DJ-I has been implicated in oxidative stress and protein folding and degradation. The pathological inclusions are commonly found in neurodegenerative diseases in susceptible population of remaining neurons and the presence of such inclusions could indicate dysfunctional cells. Intracytoplasmic Lewy body inclusion is characteristic of PD and the Lewy bodies contain α-synuclein and ubiquitin (37–41). Therefore, we stained midbrain sections from 6-month- (not shown) and 11-month-old (Fig. 6, *A* and *B*) mice with α-synuclein and ubiquitin antibodies. No α-synuclein- or ubiquitin-positive inclusions were found. Hematoxylin-eosin staining revealed no eosinophilic inclusion either (data not shown). The expression levels of α-synuclein in DJ-1 null mice were comparable with those in wild-type controls in Western blot in 5-month-old (not

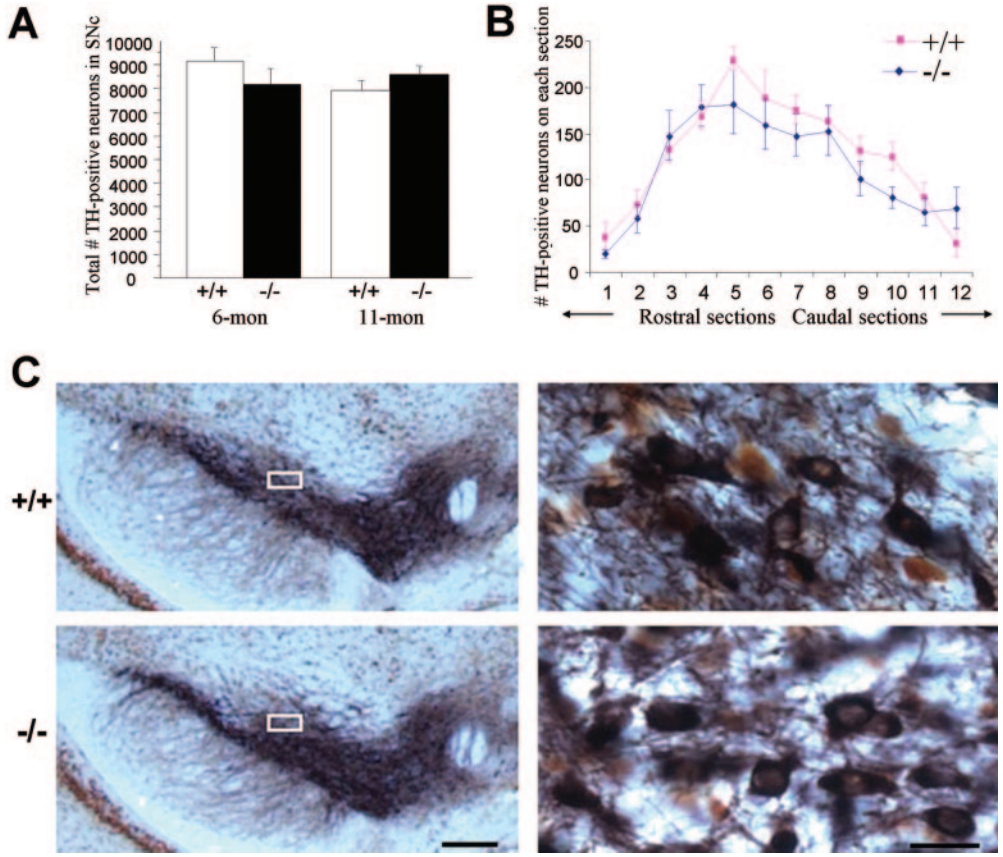

FIG. 5. **No dopamine neuron loss in SNc of DJ-1 null mice.** *A*, there was no obvious dopaminergic neuronal loss in 6-month- (*6-mon*) or 11-month-old (*11-mon*) DJ-1 null mice compared with wild-type controls ($n = 6$ for each genotype of each age). *B*, the distribution of dopamine neuron counts along the rostral-caudal axis throughout the SNc in 6-month-old mice. Similar distribution was seen in 11-month-old mice (data not shown). *C*, TH (*black*) and NeuN (*brown*) double staining of SNc in lower magnification (*left panels*) and higher magnification (*right panels*) from representative 6-month-old mice. *Scale bar* is 200 μm for the *left panels* and 20 μm for the *right panels*.

shown) as well as in 11-month-old mice (Fig. 7). Oxidative modification of proteins can be detected by antibodies against carbonylated proteins. Despite the potential role of DJ-I in oxidative stress, the absence of DJ-1 did not increase carbonyl protein levels in the brains of 5-month (data not shown) and 11-month-old mice (Fig. 7).

DISCUSSION

Since the discovery that the loss-of-function mutations in DJ-1 cause an autosomal recessive form of early-onset familial PD (1), a number of studies have been published to investigate DJ-1 function and its potential role in the pathogenesis of PD. This *in vivo* study clearly indicates that the loss of DJ-1 function in mice can lead to motor deficits as well as alterations in nigrostriatal dopaminergic function.

Interestingly, the onset of motor deficits in DJ-1 null mice is age- and task-dependent. All mice appeared healthy and seemed to live a normal life at least up to age 11 months. No motor deficits could be detected in any age groups in the rotarod test. Significant motor deficits in the open field test did not become obvious until DJ-1 null mice reached 11 months of age. Importantly, the age-dependent progression of motor deficits in DJ-1 null mice further validates the potential of this mouse model as a good model for PD, given the fact that age is the single most important risk factor in this progressive neurodegenerative disease.

In contrast, significant motor deficits could be detected in DJ-1 null mice as young as 5 months old using the tape removal task. Such task dependence is in agreement with the fact that subtle motor deficits in PD patients can be detected much earlier than the occurrence of classic motor deficits of PD (42). This could be especially true in a progressive disorder in which the affected individual could have a lengthy period to compensate for minor deficits. There have been few studies validating behavioral tasks in mice for their ability to detect mild nigrostriatal dysfunction. Tape removal may be sensitive to mild nigrostriatal dysfunction because it requires coordinated forelimb use. It is known in other rodent PD models that tasks involving forelimb use are especially sensitive in detecting subtle dopaminergic deficits (43–46). Even though the open field test is known to be sensitive to hypolocomotion often associated with hypodopaminergic function, it is not as sensitive as the tape removal task. The rotarod is commonly used in Huntington disease animal models, in cerebellum dysfunction, and in spinal cord dysfunction (47–49). However, it is a less specific and less sensitive task for dopamine system dysfunction.

It is surprising and counterintuitive that DJ-1 null mice have more tissue dopamine content, more dopamine release, as well as faster dopamine reuptake in the dorsal striatum compared with wild-type controls. The cyclic voltammetry study directly probed dopamine reuptake kinetics and the data suggested an enhancement of DAT function in DJ-1 null mice. One obvious consequence of enhanced DAT function would be elevated tissue dopamine content (due to more efficient dopamine recycling) and therefore a larger releasable pool. One relevant comparison to these conditions is the DAT knockdown mice that seem to have the opposite phenotype (50). DAT knockdown mice have decreased dopamine reuptake due to reduced DAT expression. They also have lower tissue dopamine content (due to reduced dopamine recycling) and lower dopamine release

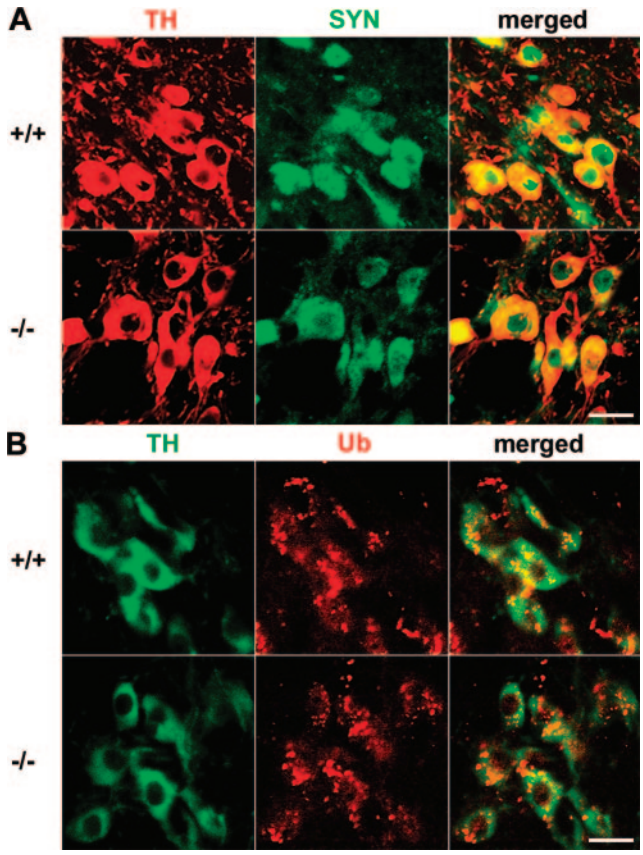

FIG. 6. **No obvious neuropathology in SNc of DJ-1 null mice.** Fluorescence immunostaining of SNc neurons for α-synuclein (*SYN*) and ubiquitin (*Ub*) in 11-month-old mice ($n = 3$ for each genotype) is shown. *A*, strong α-synuclein staining (*green*) is seen in nuclei, cytoplasm, and processes of dopaminergic neurons (labeled by TH, *red*). No inclusions or obvious expression level change were found in DJ-1 null mice. *B*, weak ubiquitin (*Ub*) staining (*red*, dot-like) is seen in both dopaminergic (labeled by TH, *green*) and non-dopaminergic cells. No inclusions or obvious expression level change were found in DJ-1 null mice. Similar results were seen in 6-month-old mice (not shown, $n = 3$ for each genotype). *Scale bar* is 20 μm.

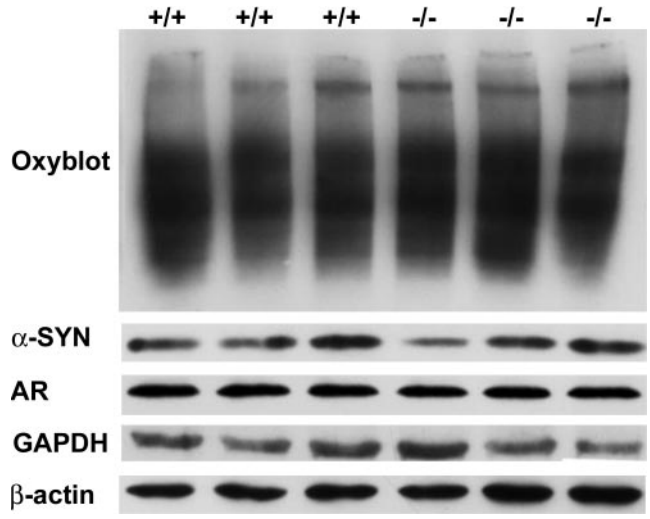

FIG. 7. **Western blot analysis of brain protein levels in 11-month-old mice ($n = 6$ for each genotype).** Protein levels for protein carbonyl (*Oxyblot*) showed comparable protein oxidation in DJ-1 null mice and wild-type controls. Western blot analysis of α-synuclein (*SYN*), androgen receptor (*AR*), and GAPDH levels did not reveal any genotype differences. Similar results were seen in 5-month-old mice (not shown, $n = 8$ for each genotype).

(due to a reduced releasable pool). Much more severe changes in the same direction were found in mice with complete loss of DAT function (51, 52). These decreases in dopaminergic parameters are the opposite of changes that we found in DJ-1 null mice. Importantly, DAT knockdown mice show hyperlocomotion in the open field, which is also the opposite of the behavioral deficit in DJ-1 null mice.

Furthermore, DAT function may be one of the most important factors that determine susceptibility to PD. First of all, higher tissue dopamine content is associated with increased cellular oxidative stress (53–58). Second, higher DAT function could confer vulnerability to neural toxins in the environment. Even though human genetic studies are still lacking, mice with no DAT expression or half of the normal DAT expression are much more resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism (59). Similarly, in *Caenorhabditis elegans*, mutations that impair DAT function make the organism less vulnerable to Parkinsonism-inducing toxins (60). Interestingly, more dopamine release and faster dopamine reuptake in DJ-1 null mice were only detected in the dorsal striatum, which is in agreement with the fact that PD affects mostly nigrostriatal DA neurons. Although we did not detect obvious elevations of DAT protein levels in DJ-1 null mice, more studies have to be conducted to determine the functional aspects of dopamine transport such as DAT compartmental expression, oligomerization, and functional modifications by DAT associated proteins (61–63).

Even though motor deficits can be detected in DJ-1 null mice as young as 5 months of age, we did not find significant dopamine neuron loss or neuropathology in DJ-1 null mice at 6 or 11 months of age. This is in contrast to human PD patients who usually develop motor symptoms only after loss of ~50–60% dopamine neurons. There are a number of possibilities that may explain the differences between human PD patients and the mouse model. First of all, it is not clear yet whether DJ-1 linked PD patients have actual dopamine neuron loss or only functional disturbances of the dopaminergic system. ^{18}F-DOPA PET scan studies have shown evidence of nigrostrial dopaminergic functional deficit in DJ-1 linked PD patients (64). However, no autopsy pathology is available yet. Second, the difficulty in detecting motor deficits in human PD patients before significant dopamine cell loss may be due to the fact that multiple behavioral compensations could happen in humans with a progressive neurodegenerative disorder. Third, neurodegenerative pathology may require a long period to manifest, whereas the life span of mice is much shorter than that of humans. Fourth, there could be intrinsic differences between mouse and human brains. For example, melanin is present in human dopaminergic neurons but not in rodents. Finally, exposure to environmental insults could be important in phenotypic expression of the genetic mutations; humans and mice are not exposed to the same type of environmental insults. Therefore, providing additional insults such as toxic exposures or other genetic mutations in the setting of DJ-1 deficiency may be necessary to manifest the full range of parkinsonian phenotypes in mouse models.

There was some suggestion of sex-specific expression of DJ-1-mediated deficits. The tape removal task showed that male DJ-1 null mice were more affected by the mutation than females. Even though one could postulate that females were not sensitive to the particular test, potential interactions between DJ-1 and the androgen receptor raise intriguing possibility of sex-specific effect of DJ-1. Earlier biochemical studies showed that DJ-1 binds to a modulator of androgen receptor PIASxa, which leads to reduced repression of androgen receptor transcription activity (15, 16). In humans, a preponderance of PD in males compared with females has been noted (65), but whether DJ-1 linked PD also has a gender bias is not known yet.

Earlier studies also found that DJ-1 was reduced in rat sperm treated with sperm toxicants that cause infertility in rats (12) and that DJ-1 may play a role in fertilization in mice (13). However, we did not see any association of DJ-1 function and male fertility in our studies; there was no change of androgen receptor levels in brains of DJ-1 null mice either (Fig. 7). Another protein that was implicated in earlier studies in relation to PD and DJ-1 was GAPDH (14, 66, 67). GAPDH was found to be co-localized with α-synuclein in Lewy bodies. It was also co-purified with DJ-1 as a complex from a rat hepatoma cell line. However, we did not see any difference in GAPDH levels between DJ-1 null mice and their wild-type controls (Fig. 7).

In conclusion, the age-dependent progression of motor deficits and neurochemical changes in the nigrostriatal pathway in DJ-1 null mice provide valuable insights on the importance of DJ-1 in the pathogenesis of PD. In addition, this model provides an unprecedented opportunity to investigate the role of aging and DJ-1-related biochemical pathways in the pathogenesis of PD. Future studies will tell us whether advanced age will eventually lead to discernable neuropathology and prominent dopamine neuron loss in DJ-1 null mice.

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Addendum—While our paper was being reviewed, the paper by Goldberg *et al*. on DJ-1-null mice appeared in *Neuron* (Goldberg, M. S., Pisani, A., Haburcak, M., Vortherms, T. A., Kitada, T., Costa, C., Tong, Y., Martella, G., Tscherter, A., Martins, A., Bernardi, G., Roth, B. L., Pothos, E. N., Calabresi, P., and Shen, J. (2005) *Neuron* **45,** 489–496) with the main findings similar to ours. They noted behavioral deficits without dopaminergic cell loss.

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