Age-dependent dopaminergic dysfunction in Nurr1 knockout mice

Chuantao Jiang\textsuperscript{a}, Xinhua Wan\textsuperscript{a,b}, Yi He\textsuperscript{a}, Tianhong Pan\textsuperscript{a}, Joseph Jankovic\textsuperscript{b}, Weidong Le\textsuperscript{a,*}

\textsuperscript{a}Parkinson Disease Research Laboratory, Department of Neurology, Baylor College of Medicine, Houston, TX 77030, USA
\textsuperscript{b}Parkinson’s Disease Center and Movement Disorders Clinic, Department of Neurology, Baylor College of Medicine, Houston, TX 77030, USA

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Abstract

The Nurr1 gene, which codes for a transcriptional factor in the nuclear receptor superfamily, plays an important role in the development of the mesencephalic dopaminergic (DAergic) system. To study the age-dependent effects of Nurr1 expression in maintaining mature nigrostriatal DAergic neuronal function, we examined motor behaviors, determined nigrostriatal dopamine (DA) levels and the number of nigral DAergic neurons, and measured the expression of several DAergic neuron-associated genes in heterozygous Nurr1-deficient (Nurr1\textsuperscript{+/−}) and wild-type mice of different ages. In contrast to the same-aged, wild-type mice, old Nurr1\textsuperscript{+/−} mice (>15 months) had a significant decrease in both rotarod performance and locomotor activities, suggesting a motor impairment that is analogous to parkinsonian deficit. Furthermore, the abnormal motor behaviors in old Nurr1\textsuperscript{+/−} mice were associated with decreased DA levels in the striatum, decreased number of DAergic neurons in the nigra, and reduced expression of Nurr1 and DA transporter in the nigra. Our data indicate that Nurr1 plays an important role in the functional maintenance and survival of nigral DAergic neurons and suggest that the Nurr1\textsuperscript{+/−} mouse is a useful animal model to study the pathogenesis of Parkinson disease (PD) and to explore disease-modifying strategies.

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Introduction

Parkinson disease (PD) is a neurodegenerative disorder affecting approximately 1\% of the U.S. population over the age of 60 (Twelves et al., 2003). The pathologic hallmarks include progressive loss of dopaminergic (DAergic) neurons in the substantia nigra (SN) and cytoplasmic inclusions called Lewy bodies (Dawson and Dawson, 2003). The decline of DA in the striatum is associated clinically with progressive bradykinesia, tremor, rigidity, and postural instability (Tedroff et al., 1999). Several animal models are currently used to study the pathogenesis and treatment of PD (Orth and Tabrizi, 2003), including 6-hydroxydopamine (6-OHDA)-lesioned rat (Glinka et al., 1997, review paper), 1,2,3,6-methyl-phenyl-tetrahydropyridine (MPTP)-lesioned mouse and primate (Jenner, 2003, review papers), and rotenone-treated rat (Betarbet et al., 2000; He et al., 2003; Lapointe et al., 2004). However, these neurotoxin-induced lesions produce acute nigrostriatal damage, after which the animals stabilize or gradually recover. Because of this, they do not mimic the progressive course of human PD (Orth and Tabrizi, 2003), greatly limiting the usefulness of these traditional models. Therefore, there is a need to develop more representative animal models of progressive PD, which can be used to study the pathogenesis of the disease and explore disease-modifying strategies.

Nurr1, a member of the nuclear receptor superfamily of transcription factors (Law et al., 1992), has been found essential for the development of ventral midbrain DAergic neurons (Saucedo-Cardenas et al., 1998; Zetterstrom et al., 1997). Previous studies have reported on the generation of knockout Nurr1 mice to assess the role of Nurr1 in the mature DAergic system and its relevance to neuronal degeneration in PD. (Castrillo et al., 1998; Saucedo-Cardenas et al., 1998; Zetterstrom et al., 1997). In Nurr1-
deficient homozygous (Nurr1−/−) mice, DAergic neurons are totally absent in the SN, and all animals die within 24 h after birth. In Nurr1-deficient heterozygous (Nurr1+/−) mice, on the other hand, the animals survive to adulthood (Le et al., 1999a; Saucedo-Cardenas et al., 1998; Zetterstrom et al., 1997). Our previous study showed that the decreased level of Nurr1 protein in Nurr1+/− mice was associated with increased vulnerability to the neurotoxin MPTP (Le et al., 1999b), providing evidence that Nurr1 plays an important role in maintaining the function of mature midbrain DAergic neurons and that a defect in Nurr1 may increase susceptibility to SN injury. Newborn Nurr1+/− mice have reduced DA levels in the striatum (Saucedo-Cardenas et al., 1998; Zetterstrom et al., 1997), but the abnormality is corrected when the animals reach adult age (Le et al., 1999b), suggesting a compensatory mechanism (Calne and Zigmond, 1991; Whone et al., 2003). Changes in locomotor activity, thought to be related to reduced mesolimbic and mesocortical DA levels (Eells et al., 2002a) without obviously altered striatal DA levels (Backman et al., 2003), have been reported in adult Nurr1+/− mice.

To investigate the effect of aging on the fate of nigral DAergic neurons in the setting of Nurr1 deficiency, we examined motor behavior, nigrostriatal biochemistry, histology, and DAergic neuron-related gene expression in old (15–24 months old) Nurr1+/− mice and compared these findings to Nurr1+/− mice of the same age and to adult (2–10 months old) Nurr1+/− mice. Our studies provide evidence linking the age-dependent changes in Nurr1 expression to progressive nigrostriatal dysfunction, thus suggesting that the Nurr1+/− mouse is a useful model for the study of progressive nigral DAergic dysfunction seen in PD.

Materials and methods

Nurr1-deficient mice

As previously described (Saucedo-Cardenas et al., 1998), Nurr1 gene disruption was generated by insertion of the neo5 gene PGKNEObpA into a unique BamHI restriction site in exon 3. The target vector was then introduced by electroporation to embryonic stem cells, and the transmission of the Nurr1 mutation was generated by microinjection into the blastocoele of blastocyst stage embryos derived from female C57BL/6 mice. Embryos were transferred unilaterally into the uterine horn of pseudopregnant F1 foster mothers. Nurr1+/− mice were mated to produce the Nurr1+/+ and Nurr1+/− offspring used in the present study. The genotype of the mice was analyzed with polymerase chain reaction (PCR) using mouse tail genomic DNA. Three primers were used in a single PCR for genotyping analysis: a 5′ primer (GGCACCCTGGTGTCTAGCTG CC) located in the 5′ end of the neo5 gene in exon 3, and two 3′ primers, one (CTGCCTT GGGAAAAGCGCCTCC) in the neo5 gene to generate a 200-bp band representing the mutated allele and the other (CAGCCCTCACAGTGCGAACAC) in a 3′ portion of exon 3 to generate a 300-bp, wild-type band. The male Nurr1+/− and Nurr1+/+ mice were separated by age group and housed in groups of four under conditions of constant temperature and controlled lighting (light period 12–12 h). All animals were fed an ad libitum diet of RMI expanded pellets and tap water.

MPTP-lesioned mice

Because MPTP administration in mice usually causes a significant loss of tyrosine hydroxylase (TH)-positive neurons in the SN and decreased DA levels in the striatum, MPTP-lesioned mice have been commonly used as a PD model (Orth and Tabrizi, 2003). In this study, we used this model as a control to compare with the behavioral changes in Nurr1+/− mice. MPTP was freshly prepared in 0.9% saline and given intraperitoneally to 3- to 4-month-old male C57BL/6 mice at a total dose of 40 mg/kg body weight in four injections at 2-h intervals. These mice were also housed in group of four under the conditions described above. Behavioral tests were performed in the mice before MPTP administration and at 12 and 30 days after administration.

Rotarod performance

Motor coordination was tested with an accelerating rotarod treadmill (Columbus Instruments, Columbus, USA), consisting of a 3-cm-diameter rotating rod raised 16 cm above a platform and divided into four chambers for testing multiple mice simultaneously. This method is designed to measure motor deficits by recording the time an animal maintains balance and keeps pace with the rotating rod (Rozas et al., 1998). Initially, each mouse was required to perch on the stationary rod for 30 s to accustom themselves to the environment. Then the animals were trained at a constant speed of 5 rpm for 90 s. After this pretraining, mice were tested three times at 1-h intervals on three consecutive days for a total of nine tests, a mean of which was undergone to statistical analysis. During each test, the rotarod was set at a starting speed of 5 rpm for 30 s, and the speed was increased by 0.1 revolution per second. The length of time that each mouse was able to maintain balance on the rotating rod was recorded with a stopwatch.

Locomotor activities

Mouse movement was monitored by the AccuScan Digiscan system (AccuScan Instruments, Inc., Columbus, USA), which employed infrared beams to detect horizontal and vertical movement. The pattern of beam breaks was computerized (VersaMax Software, AccuScan Instruments, Inc.) to generate a quantitative measure of locomotor activity. Data collected by computer included number of horizontal activities, number of vertical activities, and total distance traveled. The measurements were carried out
during the afternoon in a dark room. Each mouse was placed in the testing chamber for 30 min for adaptation, followed by a 50-min recording by the computer-generated automatic analysis system (Tillerson et al., 2002). All animals were tested three times for each experiment, and the mean of the test results was undergone to statistical analysis.

**Determination of striatal DA levels**

The levels of striatal DA were determined according to the method previously described (Le et al., 1992). Briefly, the striatum was dissected from anesthetized mice on ice-cooled plate, washed in ice-cooled phosphate buffer saline solution (PBS), and extracted by ultrasonication with 10% perchloric acid (1:10 v/v). The extracts were clarified by centrifugation at 14,000 × g for 20 min. The supernatants were filtered through acro-disc filters (mesh size 0.25 µm) and subjected to high-performance liquid chromatography (HPLC) packed with a BAS P/N reversed-phase cartridge column (Phase-II ODS 3 µm 100 × 3.2 mm). DA was detected by an electrochemical detector (BAS, Inc., West Lafayette, IN) and calibrated by an HP 3395 integrator.

**Tag-man probe real-time reverse transcription–polymerase chain reaction (RT–PCR)**

The fresh nigral tissues were dissected out under the dissecting microscope according to the mouse brain map (Franlin and Paxinos, 1996), and total RNAs were extracted with TRizol reagent (Invitrogen, Carlsbad, CA) containing 100 U DNase per ml and reverse-transcribed using iScript™ cDNA synthesis kit (Bio-Rad; Hercules, CA). One microliter of cDNA product was applied for PCR amplification. Real-time RT–PCR was carried out with the specific primers targeting mouse Nurr1, DAT, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH provided a control for equal starting amounts of total RNA in samples and PCR efficiency. The fluorescent PCR reactions were carried out in the Bio-Rad iCycler System (Bio-Rad; Hercules, CA). For Nurr1 amplification, a forward primer (5’-GAT CGA GCA GAG GAA GAC-3’)) and a reverse primer (5’- AAG GGC TCA TGT GAG CTA-3’) were used. After an initial incubation at 95°C for 3 min, the experimental reaction consisted of 40 cycles of 95°C for 15 s and 59°C for 60 s. The PCR size was 102 bp, detected by a fluorescent probe synthesized based on the sequence of mouse Nurr1 cDNA (5’-FAM CGG GCT GCA TCA CTC CTC TCC TTT AAG CAG BHQ-1 3’, Biosearch Technologies, IDT Inc., CA). For DAT amplification, a forward primer (5’-GAT CGA GCA GAG GAA GAC-3’)) and a reverse primer (5’-GAG TCT TTA TTG TGT TGA CTA-3’) were used. After an initial incubation period of 95°C for 3 min, the experimental reaction consisted of 40 cycles of 95°C for 15 s and 60°C for 60 s. The PCR size was 108 bp, detected by the fluorescent probe synthesized according to the sequence of mouse DAT cDNA (5’-fam ACC GCA GAC ACC AGT GGA GGT TCA AGA BHQ-1 3’, Biosearch Technologies, IDT Inc.). Mouse GAPDH was used as an internal control (forward primer: 5’-AAG GGC TCA TGT GAG CTA CTC TCC TTT AAG CAG BHQ-1 3’, reverse primer: 5’-AGC AGT GGA TGC AGG-3’) with the PCR size up to 100 bp detected by the fluorescent probe (5’ Texas red GCT TTC CAG AGG GGC CAT CCA BHQ-2 3’, Biosearch Technologies, CA, IDT Inc.). Fluorescent readings were analyzed quantitatively. Each experiment was conducted three times with duplicate determinations. Nurr1 or DAT transcripts quantified from real-time PCR were normalized against GAPDH transcripts.

**Immunohistochemistry studies**

As previously described (Le et al., 1999b), after anesthesia (0.1 ml per mouse of a solution containing 42.8 mg/ml ketamine, 8.6 mg/ml xylazine, and 1.4 mg/ml acepromazine), the mice were perfused through the heart with 40 ml ice-cooled PBS and then 20 ml 4% paraformaldehyde (pH 7.4). The midbrain was postfixed in 4% paraformaldehyde, protected in 30% sucrose, and then frozen in dry ice-cooled acetone. Serial frozen sections of the entire midbrain (30 µm) were cut from the rostral to the caudal end. Two adjacent sections (section pair) were systematically picked at 200-µm intervals and subjected to free-floating immunohistochemistry with TH antibody (1:2000), followed by washing, incubation with corresponding second antibody (1:300) and standard avidin–biotin immunostaining procedures (Vector Laboratories, Burlingame, CA). For unbiased cell counting, the dissector technique was used to estimate the number of nigral neurons immunoreacted with TH antibody in animal midbrain. According to the Cavalieri principle (Pakkenberg et al., 1991), the number of neurons in a specific region was estimated by multiplying the total number of neurons counted in all section pairs by 10 to reflect the total number of sections from which the section pairs were chosen.

**Statistical analysis**

Before all analyses, we confirmed data normality and homogeneity of variances. In all the values of behavioral test results, the levels of striatal DA, the number of TH-positive cells, and the relative Nurr1 or DAT expression, two- or three-factor ANOVA analysis was performed to evaluate the significance of differences between means of different groups, which was considered to be significant if P < 0.05.

**Results**

**Rotarod performance in Nurr1+/− mice**

In this and all following studies, adult mice were defined as animals between 2 and 10 months of age; old mice were
defined as animals 15 months of age or older. We found that the rotarod performance in adult Nurr1+/- mice was not significantly different from that observed in Nurr1+/+ mice of the same age (Fig. 1A). However, there was an average decrease of 32% (range 29–38%) and 39% (range 33–43%) in rotarod time for the 15- to 19-month-old and 20- to 24-month-old Nurr1+/- mice compared with the same age group of Nurr1+/+ mice, respectively; there was an average decrease of 31% (range 27–36%) and 36% (range 31–40%) in rotarod time for the 15- to 19-month-old and 20- to 24-month-old Nurr1+/- mice compared with the adult Nurr1+/- mice (Fig. 1A). MPTP-treated mice used as a positive control showed an average decrease of 35% (range 28–46%) in rotarod time at 12 days after MPTP treatment, corresponding to the occurrence of maximal neurotoxicity, and an average decrease of 18% (range 12–27%) in rotarod time at 30 days after MPTP treatment, indicating a partial recovery in motor deficits as time progresses (Fig. 1B).

Locomotor performances in Nurr1+/- mice

All measurements of locomotor performances (horizontal movements, vertical movements, and total distance) were significantly increased by 29–48% in the adult Nurr1+/- mice, as compared with the same age group of Nurr1+/+ mice and by 31–59% as compared with the adult Nurr1+/- mice. MPTP-treated mice used as a positive control showed a significant increase in all three measurements of locomotor performance in the old Nurr1+/- mice compared with the control Nurr1+/- mice of the same age (Fig. 2A). However, after Nurr1+/- mice reached 15 months or older, the locomotor performances in all measurements were significantly decreased by 27–50% as compared with the same age group of Nurr1+/+ mice and by 31–59% as compared with the adult Nurr1+/- mice (Fig. 2A), suggesting age-related progressive motor deficits. MPTP treatment in adult C57BL/6 mice resulted in...
significant increase in horizontal movements and total distance (Fig. 2B), which was similar to that observed in the adult \( \textit{Nurr1}^{+/-} \) mice.

**Nigral TH-positive neurons in \( \textit{Nurr1}^{+/-} \) mice**

The number of TH-positive DAergic neurons in the SN of \( \textit{Nurr1}^{+/-} \) and \( \textit{Nurr1}^{+/-} \) mice at different ages was determined with an unbiased stereological optical dissector according to the Cavalieri principle (Pakkenberg et al., 1991). As shown in Fig. 3, there was no significant decrease in the number of nigral TH-positive neurons in the adult \( \textit{Nurr1}^{+/-} \) mice compared with the same age group of \( \textit{Nurr1}^{+/-} \) mice (Figs. 3Aa, d). With increased age, there was a gradual and significant loss of TH-positive cells in the SN of \( \textit{Nurr1}^{+/-} \) mice, resulting in a 28% (range 22–34%) loss at 15–19 months old (Figs. 3Ab, e) and a 37% (range 31–43%) loss at 20–24 months old (Figs. 3Ac, f) compared with the same age group of \( \textit{Nurr1}^{+/-} \) mice (Fig. 3B). As compared with the adult \( \textit{Nurr1}^{+/-} \) mice, the 15- to 19-month-old \( \textit{Nurr1}^{+/-} \) mice lost 23% (range 18–29%) and the 20- to 24-month-old \( \textit{Nurr1}^{+/-} \) mice lost 31% (range 27–38%) of TH-positive cells in the SN (Fig. 3B).

**Striatal DA levels in \( \textit{Nurr1}^{+/-} \) mice**

The levels of DA in the striatum as measured by HPLC showed no significant decrease in the old \( \textit{Nurr1}^{+/-} \) mice compared with the adult \( \textit{Nurr1}^{+/-} \) mice, and no significant difference between the adult \( \textit{Nurr1}^{+/-} \) and the same age group of \( \textit{Nurr1}^{+/-} \) mice. However, the striatal DA levels were significantly decreased in the old \( \textit{Nurr1}^{+/-} \) mice, which showed a 32% (range 26–38%) decrease in the 15- to 19-month-old mice, and a 42% (range 35–51%) decrease in the 20- to 24-month-old mice compared with the \( \textit{Nurr1}^{+/-} \) mice of the same age (Fig. 4). As compared with the adult \( \textit{Nurr1}^{+/-} \) mice, the striatal DA levels in the 20- to 24-month-old \( \textit{Nurr1}^{+/-} \) mice was significantly decreased by 44% (range 36–55%; Fig. 4).
and months old also showed significant decrease (*P < 0.01 for 15- to 19-month-old Nurr1+/− mice and **P < 0.01 for 20- to 24-month-old Nurr1+/− mice as compared with the same age group of Nurr1+/+ mice. Each group consisted of six to eight mice. There was no significant decrease in the striatal DA levels as compared with the adult Nurr1+/+ mice.

Fig. 4. DA levels in the Nurr1+/+ and Nurr1+/− mice of 2–10 months old (a), 15–19 months old (b), and 20–24 months old (c). Each group consisted of six to eight mice. There was no significant decrease in the striatal DA levels with *P < 0.05 for 15- to 19-month-old Nurr1+/− mice and **P < 0.01 for 20- to 24-month-old Nurr1+/− mice as compared with the adult Nurr1+/+ mice.

Nurr1 and DAT mRNA expression in the Nurr1+/− mice

We measured both Nurr1 mRNA levels and DAT mRNA levels in the nigra of Nurr1+/− and Nurr1+/+ mice using real-time RT–PCR. There was a marked decrease of Nurr1 mRNA expression in the old Nurr1+/− mice with 42% (range 35–53%) reduction compared with the same age group of Nurr1+/+ mice and 41% (range 33–52%) reduction compared with the adult Nurr1+/− mice (Figs. 5B, E). There was no significant difference in Nurr1 mRNA expression between the adult and old Nurr1+/+ mice, and between the adult Nurr1+/+ mice and the Nurr1+/− mice of the same age, respectively (Figs. 5B, E). DAT mRNA levels were not significantly decreased in the adult Nurr1+/− mice compared with the adult Nurr1+/+ mice but were significantly decreased by 35% (range 30–42%) and 30% (23–37%) in the old Nurr1+/− mice compared with the old Nurr1+/+ mice and the adult Nurr1+/− mice, respectively (Figs. 5D, F). Taken together, the data indicate that Nurr1 may regulate DAT mRNA expression, leading to lower DAT mRNA levels in the old Nurr1+/− mice.

Discussion

Age-related decrease in Nurr1 expression leads to progressive DAergic dysfunction

PD is characterized by progressive loss of SN neurons associated with low DA levels in the striatum. The development of nigral DAergic neurons and the maintenance of DAergic function are critically dependent on the function of Nurr1, a transcription factor in the nuclear receptor superfamily (Le et al., 1999a; Saucedo-Cardenas et al., 1998). In agreement with our findings here, several studies have shown that altered central DAergic transmission is associated with increased spontaneous locomotor behaviors in adult Nurr1+/− mice (Backman et al., 2003; Eells et al., 2002a). Eells et al. (2002a) measured DA levels in midbrain, striatum, nucleus accumbens, and prefrontal cortex in adult Nurr1+/− mice and reported that mesolimbic and mesocortical DA levels were reduced in Nurr1+/− mice, in association with the increased stress-induced locomotor activity. In a second behavioral study, Backman et al. (2003) suggested that elevated locomotor activity in adult Nurr1+/− mice might be related to the alteration in DA release and uptake within the nigrostriatal system.

Based on these results, we studied old Nurr1+/− mice to determine whether aging may exaggerate the altered central DAergic transmission and compromise the compensatory mechanism that appears to work in younger Nurr1+/− animals. We found that locomotor behaviors in the old Nurr1+/− mice were significantly decreased compared with the adult animals, suggesting that when the animals reach advanced age, their normal compensatory mechanisms fail to maintain the nigral DAergic function as a result of Nurr1 deficiency (Calne and Zigmond, 1991; Tedroff et al., 1999; Whone et al., 2003). Therefore the old Nurr1+/− mice develop behavioral, biochemical, and pathological changes relevant to PD.

Rotarod performance also showed a progressive motor deficit in the old Nurr1+/− mice (Fig. 1A). This was similar to the motor deficit found in MPTP-lesioned mice, but in contrast to the Nurr1+/− mice, the MPTP-lesioned mice gradually recovered (Fig. 1B). Furthermore, the behavioral changes, as demonstrated by locomotor activities and rotarod performance, continued to worsen as Nurr1+/− mice aged (Fig. 2). The motor impairment in old Nurr1+/− mice seems to correlate with the loss of nigral TH-positive neurons, reduction in the striatal DA levels, and decreased DAT mRNA expression. Activated by Nurr1 during development (Saucedo-Cardenas et al., 1998; Smits et al., 2003; Zetterstrom et al., 1997), these phenotypic markers of the DAergic system markedly decrease after age 15 months. While there were no significant alterations in the number of TH-positive SN neurons in striatal DA levels or in the DAT mRNA levels of nigral tissues in either the adult Nurr1+/− mice or the old Nurr1+/− mice, we showed a precipitous drop in these markers of DAergic function in the old Nurr1+/− mice. This supports the notion that the DAergic system is particularly susceptible to the influences of aging, possibly as a result of age-related decrease in Nurr1 expression. Although reduced Nurr1 expression does not affect the number of nigral DA neurons in adult Nurr1+/− mice (Eells et al., 2002b; Le et al., 1999b), we postulate that when Nurr1 expression is reduced below a critical threshold,
as occurs in old Nurr1+/− mice, particularly when coupled with some endogenous factors (e.g., increased oxidative stress, increased excitotoxicity, abnormal mitochondrial function) and exogenous environmental factors (e.g., exposure to toxins), the normal maintenance of DA function is adversely altered, leading to motor deficits.

In human brains, the number of midbrain DAergic neurons is reduced by 4.7–6.0% per decade after the age of 50 (Fearnley and Lees, 1991; Gibb and Lees, 1991), but this cell death is not enough to produce parkinsonian symptoms until a critical level of 60% to 80% loss is reached (Lee et al., 2000). While the mechanisms for age-related nigral neuron degeneration in PD are unknown, it is possible that an age-related decrease in Nurr1 expression increases the vulnerability of the SN neurons to environmental stress. In support of this, Chu et al. (2002) demonstrated that the loss of Nurr1 immunoreactivity in human SN is highly correlated with the decline in DAergic neurons in the brains of aging. This is consistent with our previous finding that low levels of Nurr1 protein made Nurr1+/− mice more vulnerable to the neurotoxin MPTP compared to wild-type mice (Le et al., 1999b). Working with primary mesencephalic cultures, Eells et al. (2002b) showed that deletion of a Nurr1 allele resulted in reduced survival of DAergic neurons. A recent study by Chu et al. (2003) in 12 clinically defined and pathologically proven cases of PD and 8 age-matched non-PD controls showed a significant decrease in Nurr1 immunoreactivity in nigral neurons of PD brains compared with control brains (58.1% decrease, \( P < 0.01 \)). Furthermore, double-labeling studies found that Nurr1 was not expressed in nigral neurons containing \( \alpha \)-synuclein, a marker of both sporadic and familial PD, possibly indicating that decreased expression of Nurr1 plays a role in the pathogenesis of nigral DA neuronal degeneration in PD (Chu et al., 2003). An interaction between \( \alpha \)-synuclein and Nurr1 is also sug-
gested in a study by Baptista et al. (2003), who found reduced expression of Nur1 in neuroblastoma cell line transfected with α-synuclein. Down-regulation of the Nur1 gene may also result in reduced synthesis of DAT and vesicular monoamine transporter-2 (Hermanson et al., 2003). This in turn could interfere with the axonal transport of α-synuclein to terminals and accumulation of α-synuclein in the cell body (Le et al., unpublished data). We are currently investigating the effects of reduced Nur1 expression on neuronal and glial α-synuclein in models of human synucleinopathies.

The Nur1+/− mouse model is a useful animal model to study progressive PD

Understanding the pathogenesis of PD would aid in developing better treatments including disease-modifying strategies. Current animal models of PD, including MPTP-treated mice and rotenone-treated rats, are associated with acute toxicity resulting in rapid apoptosis of some midbrain DAergic neurons. Furthermore, neither model shows evidence of progressive neurodegeneration. Thus, MPTP treatment induced PD-like locomotor impairment, but this abnormal behavior recovered gradually after 30 days (Fig. 1B). Chronic administration of rotenone in rats can cause some DAergic neuronal degeneration and putative inclusion body formation (Betarbet et al., 2000), but the effects of rotenone toxicity are widespread, relatively nonspecific, and nonprogressive (Lapointe et al., 2004). Administration of the herbicide paraquat in combination with the fungicide rotenone toxicity are widespread, relatively nonspecific, and nonprogressive (Lapointe et al., 2004). Administration of the herbicide parapquat in combination with the fungicide mecoprop to C57BL/6 mice results in an accumulation of toxicity effects in the nigrostriatal DAergic system, but the toxicity appears to persist only in the old mice (Thiruchelvam et al., 2003). Furthermore, genetically modified animals (including knockout of the α-synuclein, parkin, or UCHL1 genes in mice) have not developed parkinsonism, and overexpression of either mutant or wild-type α-synuclein gene in mice is not associated with obvious nigral DA neuron degeneration (Le and Appel, 2004). On the other hand, several studies using transgenic Drosophila over-expressing wild-type or mutant α-synuclein have found mild to moderate nigral degeneration (Auluck et al., 2002).

Overall, the models described to date still fall short of replicating the progressive pathological and clinical features of the human disease. In contrast to the traditional models, Nur1+/− mice display an age-dependent DA dysfunction associated with motor decline, loss of DAergic neurons, reduction in nigrostriatal DA levels, and loss of Nur1 and DAT expression. These findings provide behavioral, biochemical, and pathological evidence that the Nur1+/− mouse may serve as a model of progressive disease with features similar to those observed in human PD. As such, this model can be used not only to study the pathogenesis of the disease, but also for testing drugs with potential neuroprotective or disease-modifying effects.

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