Pharmacology, Biochemistry and Behavior 99 (2011) 324–332

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Aged monkeys as a partial model for Parkinson's disease

P.J. Hurley [a], J.D. Elsworth [a], M.C. Whittaker [b], R.H. Roth [a], D.E. Redmond Jr. [a,*]

[a] Departments of Psychiatry, Neurosurgery, and Pharmacology, Yale University School of Medicine, 300 George Street 9th Floor, New Haven, CT 06510, USA
[b] St. Kitts Biomedical Research Foundation, St. Kitts, St. Kitts and Nevis

ARTICLE INFO

Article history:
Received 5 March 2011
Received in revised form 5 May 2011
Accepted 10 May 2011
Available online 18 May 2011

Keywords:
Aging
Parkinson's disease
African Green monkey
Dopaminergic decline
MPTP
Dopamine agonists
L-Dopa
Apomorphine
Dihydrexidine
Pergolide

ABSTRACT

Parkinson's Disease (PD) and the natural aging process share a number of biochemical mechanisms, including reduced function of dopaminergic systems. The present study aims to determine the extent that motor and behavioral changes in aged monkeys resemble parkinsonism induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The behavioral and physiological changes in PD are believed to result largely from selective depletion of dopamine in the nigrostriatal system. In the present study, ten aged female monkeys were compared with three groups: 9 untreated young adult female monkeys, 10 young adult male monkeys and 13 older male monkeys that had been exposed to MPTP. Trained observers, blind as to age and drug condition and without knowledge of the hypotheses, scored the monkeys using the Parkinson's factor score (*Parkscore*), which has been validated by a high correlation with post mortem striatal dopamine (DA) concentrations. The aged animals had higher scores on the *Parkscore* compared with the young adults, with most of its component behavioral items showing significance (*tremor*, *Eating Problems*, *Delayed initiation of movement*, and *Poverty of Movement*). L-Dopa and DA-agonists did not clearly reverse the principal measure of parkinsonism. DA concentrations post mortem were 63% lower in 3 aged monkeys in the ventral putamen compared with 4 young adults, with greater reductions in putamen than in caudate (45%). We conclude that aged monkeys, unexposed to MPTP, show a similar profile of parkinsonism to that seen after the neurotoxin exposure to MPTP in young adult monkeys. The pattern of greater DA depletion in putamen than in caudate in aged monkeys is the same as in human Parkinson's disease and contrasts with the greater depletion in caudate seen after MPTP. Aged monkeys of this species reflect many facets of Parkinson's disease, but like older humans do not improve with standard dopamine replacement pharmacotherapies.

1. Introduction

Parkinson's Disease (PD) is characterized by a progressive degeneration of motor function, tightly linked to dopaminergic neuron death in the substantia nigra. As the brain's dopaminergic functions diminish with the progression of the disease, many symptoms emerge, such as bradykinesia, rigidity, motor freezing, resting tremor, difficulty in initiating movement, postural instability, incoordination, and difficulty with speech and swallowing, which eventually render the patient unable to carry out normal motor functions (Hoehn and Yahr, 1967). Although the precise cause of PD is unknown in most cases, there are a variety of risk elements associated with the disease and the main factor is the age of the patient. More than half of people over the age of 85 display parkinsonian symptoms and signs whether or not they are diagnosed with the disease (Bennett et al., 1996). Depletion of the dopaminergic system and the observable and measurable decrease in motor function seen in PD are both major features of normal aging in humans (Adler et al., 2002; Adolfsson et al., 1979; Agid et al., 1996; Calne and Peppard, 1987). One important difference between aging and PD is the rate of the progressive decline that is observed. Normal aging sees declines in the nigrostriatal DA neurons of 4.7% per decade, or 33% between age 20 and 90 (Fearnley and Lees, 1991), whereas PD requires nigrostriatal losses of at least 50% and dopamine depletions of at least 80% for signs to appear (Fearnley and Lees, 1991; Marsden, 1990). Other biochemical factors are shared between the processes of PD and aging. Introducing a point mutation in the α-synuclein gene produces a crucial protein component of Lewy bodies, intra-neuronal aggregates that are associated with PD etiology. In these transgenic mice this abnormality can lead to consistent age-related declines in dopaminergic function sufficient to induce parkinsonian signs (Plaas et al., 2008). Similar results ensue when heterozygote knockouts for the Nurr1 transcription factor are compared behaviorally and biochemically to aged-matched wild-type counterparts (Jiang et al., 2005). Similarly, genetic reductions of the GDNF receptor, which aids in the survival and proliferation of dopamine neurons in normal development and after neurotoxic events, have been shown to contribute to age-related declines in the dopaminergic system in partial knockout mice (Zaman et al., 2008). Mitochondrial DNA depletions have been implicated in the neurodegenerative loss in both aged and PD patients (Bender et al., 2006). Recent evidence indicates that several markers of

* Corresponding author. Tel.: +1 203 785 4432; fax: +1 203 776 2893.
E-mail address: Eugene.Redmond@Yale.edu (D.E. Redmond).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.05.007

risk factors for DA neuron degeneration in PD also are expressed in aging monkeys in those DA neurons that are vulnerable to degeneration, including changes in the distribution of alpha-synuclein (Chu and Kordower, 2007), function of the proteasome and lysosome systems (Kanaan et al., 2007), accumulation of oxidative damage (Kanaan et al., 2008a), and the presence of activated microglia (Kanaan et al., 2008b). In spite of these shared factors, PD has been described as accelerated aging that is perhaps sparked by some acute event (Hawkes, 2008). It is clear that what has been called "simple aging" does not normally qualify as PD. What is important is to determine the line at which the divide between "normal" and "abnormal" aging of the nigrostriatal DA system (Parkinson's disease) exists and to determine what biochemical and behavioral factors contribute to the placement of this line. Others have suggested a combination of environmental events and genetic variations which combine with the alterations produced by aging (Collier et al., 2007).

One of the difficulties in studying PD in animal models has been replicating the progressive and continuous decline in dopaminergic function that is observed in clinical cases of the disorder. It has been difficult to induce a sustained and progressive decline in dopaminergic function after acute treatment with neurotoxins. Nonetheless, the most common models used for PD have been the 6-hydroxydopamine (6-OHDA)-lesioned rat (Bjorklund and Stenevi, 1979; Kaakkola and Teravainen, 1990; Ungerstedt, 1971) and the unilaterally (Bankiewicz et al., 1986; Guttman et al., 1990; Kurlan et al., 1991b) or bilaterally-lesioned primate after exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Deutch et al., 1986; Jacobowitz et al., 1984; Mendez and Finn, 1975; Redmond et al., 1986; Tetrud et al., 1986). MPTP has been shown to disrupt the nigrostriatal DA pathway in monkeys, and observations of MPTP-treated *Chlorocebus sabaeus* (African green) monkeys have shown that the nigrostriatal DA depletions induced by the initial neurotoxic treatment can be sustained consistently over a long period of time, although the severity of the induced changes has been shown to vary from animal to animal (Redmond, in press; Taylor et al., 1994; Taylor et al., 1997). Recently data collected over a 10 year period after initial acute MPTP exposure suggests that small increases in parkinsonism occur over time (Redmond, in press). Behavioral changes induced by MPTP exposure in this species are reversed in part by intrastriatal grafting with fetal dopamine precursor cells (Elsworth et al., 1996; Elsworth et al., 1989a; Elsworth et al., 1989b; Redmond et al., 1986; Sladek et al., 1988; Taylor et al., 1991) or with human neural stem cells (Redmond et al., 2007) (see reviews (Anisimov, 2009; Fitzpatrick et al., 2009)).

Several studies have used aged monkeys as a partial model for Parkinson's disease, since aged monkeys are "on the threshold of expression of parkinsonian features," (Collier et al., 2007). Reduced striatal dopamine concentrations have been noted previously in squirrel monkeys (Irwin et al., 1994) and fewer tyrosine hydroxylase expressing neurons in the substantia nigra were found in aged rhesus monkeys (Emborg et al., 1998) but not in squirrel monkeys (Irwin et al., 1994; McCormack et al., 2004). Additionally, the administration of both dopaminergic precursors and growth factors, such as GDNF, have shown promise in *in vivo* experiments in aged monkeys(Emborg et al., 2009; Grondin et al., 2000; Kordower et al., 2000). These studies have tracked "improvement" in primate motor function with measures of fine motor movement, general activity, and "clinical" rating scales, which measure some characteristics of parkinsonism and correlate with the density of tyrosine hydroxylase positive neurons, as well as dopamine transporter labeled neurons (Emborg et al., 1998). Other studies reported improvements in upper limb function and motor slowing after L-Dopa in aged rhesus monkeys (Grondin et al., 2000; Kurlan et al., 1991a; Zhang et al., 2000). Although some of these methods are highly quantitative and objective, they do not address the full spectrum and characteristic changes of Parkinson's disease. Motor movement tracked with computers or video, for example, may not be selective of the unique combination of signs resulting from Parkinson's disease, and there is no generally accepted single method of diagnosing or assessing the progression of Parkinson's disease.

To obtain a different and more comprehensive assessment of the unique cluster of behavioral and motor changes in Parkinson's disease, (Redmond, in press; Redmond et al., 1986; Taylor et al., 1994) we empirically developed and validated an assessment method that utilizes classic characteristics of human PD and includes quantitative measures of species typical primate behaviors. Useful aspects of this Parkinson's rating scale are its high correlations with nigrostriatal DA concentrations, and the fact that the overall score reflects the aspects of the disease that are unique for parkinsonism. Using this scale, we determined whether aged St. Kitts green monkeys show typical signs of Parkinson's disease, how they compare with changes in MPTP-treated monkeys, and whether they respond to direct or indirect DA agonists with known antiparkinsonian effects.

2. Materials and methods

The present study compared groups of non-toxin-exposed aged monkeys both with younger monkeys, and with monkeys rendered

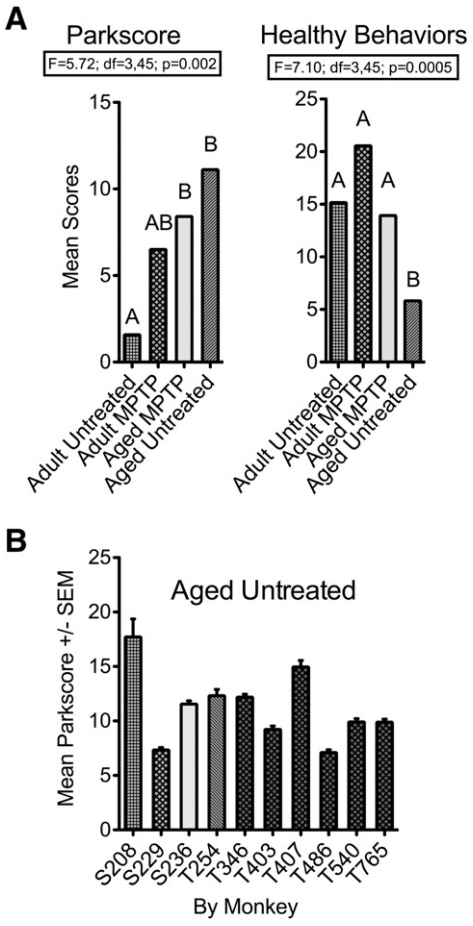

Fig. 1. Comparison of *Parkscore* and *Healthy behavior* factor scores in Aged, Untreated, and MPTP exposed monkeys. Fig. 1A. The "Aged Untreated monkeys" showed *Parkscores* that were not different from "Aged MPTP" and mildly parkinsonian "Adult MPTP"-lesioned monkeys, and increased scores as compared with "Adult Untreated" monkeys. *Healthy behaviors* (similar to "Activities of Daily Living") were reduced in the aged animals as compared with the "Adult Untreated" monkeys. Identical letters on the bars indicate that there were no differences between them (Student Newman–Keuls test, $p<0.05$). Different letters were statistically different. The inset boxes show the results of the between group analysis of variance.) Fig. 1B. Individual *Parkscores* of the Aged Untreated monkeys show the variation in scores within this group. Although two monkeys had a higher level of severity, there was no suggestion that the group effect was due to a few individuals.

mildly parkinsonian by MPTP. All animal work complied with NRC guidelines and was approved by the relevant animal care and use committees.

2.1. Selection and care of monkeys for study

The monkeys were trapped or colony bred *C. sabaeus* from the island of St. Kitts, West Indies. Throughout their time in captivity, the monkeys were fed recommended amounts of Harlan Teklad NIB Primate Diet (No. 8773, 20% protein, Madison, WI) supplemented with locally grown fruits, given unrestricted access to water, and maintained in semi-outdoor enclosures that allowed ambient natural daylight at 17′ North latitude. Four groups were selected for the studies. Ten female monkeys (ages 21–30 years) were selected for the "Aged Untreated" group with age based upon birth dates, or estimated from time of captivity plus 2 to 5 years based upon weight and size at the time of capture. Nine female monkeys (ages 5–7) were used as normal "Adult Untreated" controls. Thirteen male monkeys (ages 10–24) had been exposed to MPTP at least eight years before the data were collected for this study ("Aged MPTP" group). Ten young adult male monkeys (ages 5–6) recently exposed to MPTP composed the final group ("Adult MPTP"). The lifespan of these monkeys in captivity is about 30 years, and the females seldom have infants after they are 18 years old.

2.2. Behavioral observation methods

Trained, blinded observers 'scored' and 'rated' the behavior and motor movements of each monkey individually without knowledge of the experimental variables. Signs of advanced age in primates are sufficiently obvious to unblind observers, as well as those of moderate to severe effects from MPTP. The observers, however, had no hypotheses regarding the outcomes and carried out observations in a setting with multiple on-going studies. From these quantitative assessments of 29 behaviors, a parkinsonian summary score (*Parkscore*) was derived, based on a principal component factor analysis of a dataset of observations of 11 normal and 66 post-MPTP monkeys (See Taylor et al., 1994, for detailed operational definitions of the behaviors and factor derivations and validation). *Parkscore* validity has been tested by a number of methods and is highly responsive to pharmacological changes in DA function and correlates highly with striatal DA concentrations post-mortem (Elsworth et al., 2000; Redmond, in press). In addition to the *Parkscore*, a number of other factor scores were derived from these observations and were analyzed. A "*Healthy behavior*" summary score, consisted of the sum of several factors representing most normal behaviors that are a part of a monkey's daily activities (Redmond, in press; Taylor et al., 1994). This sum factor is equivalent to the "activities of daily living measure" that is one section of the Unified Parkinson's Disease Rating Scale (UPDRS) commonly used in clinical studies (Fahn et al., 1987; Martinez-Martin et al., 1994). This score moves inversely with the *Parkscore* (Taylor et al., 1994). It is less specific for parkinsonism than the *Parkscore*, and can be depressed by other illnesses or drugs that do not directly affect Parkinson's signs or symptoms. Severity levels described were determined by the ranges from quintiles of *Parkscore* severity from a large number of monkeys exposed to the same doses of MPTP (Redmond, in press; Taylor et al., 1994).

Table 1
Brief definitions of behaviors scored by time or rated by severity.

Individual observed behaviors score 1 or 0 per five-seconds or per five-seconds duration	
Bipedal lookout	Standing on legs with hands not touching the floor and gazing outside of cage.
Cage pick	Manual exploration or manipulation of any part of the cage.
Chew/Bruxism	Alternating mandibular-maxillary apposition without previous introduction of food or objects into mouth.
Drinking	Contact between mouth and drinking spout.
Eating	Actively gathering, manipulating, introducing into the mouth, chewing, and swallowing of food.
Eyes closed	Motionless (as defined below) with eyelids closed for five-seconds
Facedown	Lying down in contact with the cage floor or perch.
Freeze/Motionless	Remaining motionless for five-seconds duration
Penile erection	Protrusion or raising of the penis
Scratch	Rapid and repeated rubbing of any body part with fingers, hand, or foot
Self groom	Gentle manipulation of the monkey's own hair or skin with hands or mouth
Shift	Pacing or walking about the cage
Tail flag	Holding the tail erect while standing or walking
Threaten outside	Prolonged stare at observer or monkeys in other cages
Vertical climb	Locomotion on sides or top of the cage
Vocalization	Any pharyngeal or laryngeal sounds
Behaviors scored at end of observation period after food presentation or vocal threat (scored from 0 to 5)	
Food response	Speed with which animal reaches for, handles, and eats segment of banana or other highly prized fruits
Delayed movement	Degree to which motor movement appears to be delayed although eventually carried out
Difficulty eating	Physical difficulty in handling, biting, chewing, or swallowing food or liquids
Appearance	General condition of grooming and appearance (Scored 0 for normal to 5 very abnormal). This is not a measure of "blank" facies seen in parkinson's patients.
Poverty of movement	Slowness, decreased complexity, and small quantity of movement
Threat response	Motor, facial, and vocal responses to postural and vocal threats from humans
Head tremor	Oscillating movement of head at rest or during "voluntary" movement
Limb tremor	Oscillating movement of any limbs at rest or during "voluntary" movement
Effect of "intention"	*Tremor* observed and scored either decreases (negative value from 0 to 5) or increases (positive value from 0 to 5) with "voluntary" movements.
Spontaneous freeze	Interruption of an on-going motor movement pattern of any type which lasts at least five-seconds
Behavior summaries derived from factor analyses (These behaviors are derived by summing the primary behaviors scored):	
Parkscore	The sum of the following individual scores, as defined below: Head tremor, limb tremor, appearance, freeze/motionless/5, difficulty eating, delayed initiation of movement, poverty of movement, response to threat, facedown
Tremor	Head tremor, limb tremor
Anxiety	Yawn, Chew, Scratch, Self-groom, Penile erection
Arousal	Shift, tailflag, bipedal lookout, vertical climb
Sedation	Eyes closed and freeze
Quiet OK	Self-groom, cage pick, eating, drinking
Healthy	Arousal, anxiety, quiet OK

The timing of behavioral observation differed based on the treatment being assessed. Most observations of animals without drug treatments occurred twice during the same time period each day two or three times/week for several months, while the timing of observations of animals treated with dopamine agonists such as L-Dopa or pergolide was based on pharmacokinetics and pharmacodynamics of the drug and preliminary observations.

2.3. Data handling and analysis

Daily observations were recorded on a paper check sheet. The resulting data were coded into a Microsoft Excel file and checked against the paper records by the individuals who did the observation. These files were then analyzed statistically (SAS Institute I, 1988).

The factor scores were constructed, and data were tested for variance homogeneity and distribution assumptions. Multifactor parametric ANOVA for between group and repeated measures within group was performed first. Significant interactions among factors were decomposed using the simple main effects. Post hoc tests used Student-Newman–Keuls, at $p<0.05$ two tailed.

2.4. MPTP and other drug exposures

MPTP was administered intramuscularly to the MPTP comparison groups over a period of five days, totaling a dose of 2 mg/kg of body weight. Four doses were administered with a spacing of approximately 12 h between each dosing for the first three days, with the fifth dose being administered on the morning of the fifth day. The "Aged Untreated" monkeys were retired breeders, were not administered any experimental drugs, and received only standard drugs used in a primate colony, including ketamine 10 mg/kg, xylazine 2 mg/kg, for routine care every three months, immunizations, TB testing, and periodic ultrasound determinations of pregnancy.

2.5. Drug challenges

Responses to challenges with dopamine receptor agonists and precursors were measured. The challenges were administered to monkeys in the "Adult Untreated" and "Aged Untreated" groups, except for pergolide, which was administered to the "Aged Untreated" group and compared with the responses of the "Adult MPTP" group. The "Aged MPTP" group was studied behaviorally and sacrificed for another study before the drug testing was carried out. The drugs administered included L-3,4-dihydroxy-phenylalanine (L-Dopa) methyl ester with injection 1 h beforehand with the peripheral decarboxylase inhibitor, benserazide (20 mg/kg i.m.) for four days at a time with behavioral observation 3 h after L-Dopa administration (5–10 mg/kg i.m.), dihydrexidine (1 mg/kg i.m. for two days in the span of a week), and apomorphine (0.2 mg/kg i.m. for two days in the span of a week. Domperidone 3 mg/monkey was administered orally in banana 2 h before apomorphine administration to minimize or eliminate nausea or vomiting. Pergolide (0.05–0.1 mg/kg/day i.m.) was administered in increasing doses over a period of 17 days. Drug challenges were compared between the groups and against extensive baseline observations. Since the effective anti-parkinson's doses of dihydrexidine, apomorphine, and L-Dopa had been previously determined, we compared these compounds in normal "Adult Untreated" monkeys to determine side effects and effects of administration. With pergolide, we used the "Adult MPTP" group to verify anti-parkinson's effects of the doses studied. The group names "Aged Untreated" and "Adult Untreated" refer to whether the animals had been exposed to MPTP. Both of these groups as well as the "Adult MPTP" group received drug challenges.

3. Results

3.1. Behavioral differences between aged and control monkeys

The "Aged Untreated" monkeys showed a significantly higher *Parkscore* than their "Adult Untreated" control group (see Fig. 1 and Table 1 for definitions of behaviors and factor scores). Their level of parkinsonism was comparable to the "Aged MPTP" and the "Adult MPTP" monkeys. *Healthy behavior* summary score was not significantly reduced compared with normal and MPTP-lesioned adult controls. The "Aged Untreated" monkeys displayed significant deficits in the "*delay*" and "*Poverty of Movement*," and also showed *Eating Problems* that are common signs of PD (see Fig. 2). Resting or intention *tremors* were not, however, statistically higher in the "Aged Untreated" group than in the "Adult Untreated" group. Lower scores for *anxiety* (*yawn, chew, scratch, self-groom, penile erection*) and *arousal* factor scores (*shift, tailflag, bipedal lookout, vertical climb*) in the "Aged Untreated" group did not change the overall *healthy behavior* of the monkeys significantly compared to controls (see Fig. 3). We

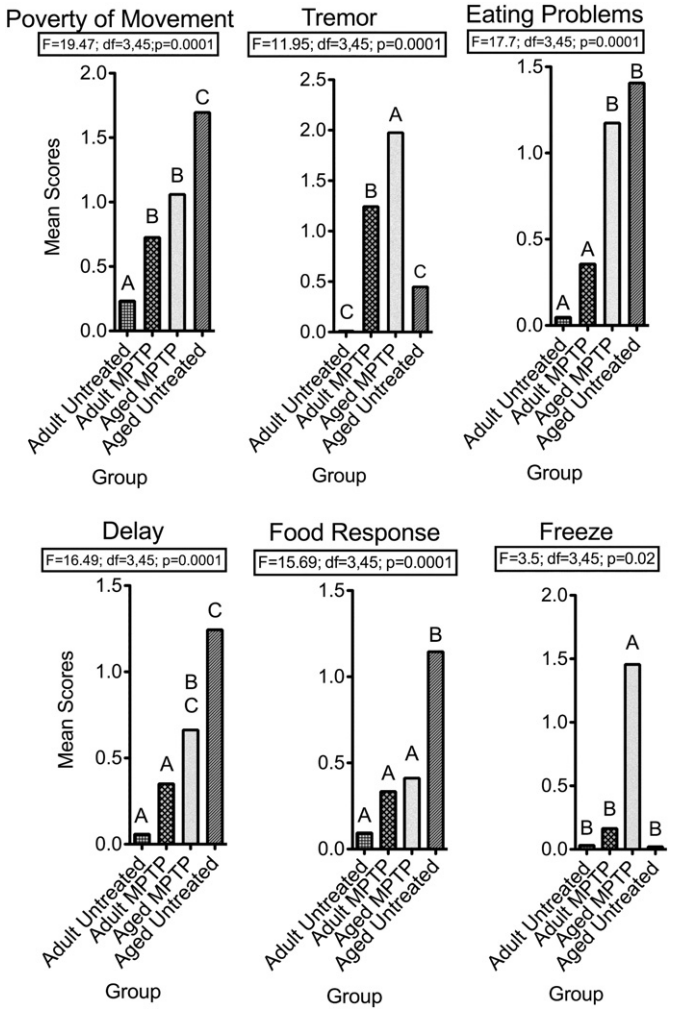

Fig. 2. Behavioral differences in specific component scores among the four groups. The aged monkeys showed effects similar to MPTP-lesioned adult monkeys. In particular, scores such as *Poverty of Movement, Delayed initiation of movement, Eating Problems,* and *Food Response* were significantly different and impaired compared with the other groups. The MPTP exposed groups all showed more tremor than the "Aged Untreated". The letters and statistical notations are the same in all of the figures. Behaviors, not illustrated but indicating significant group differences, were *vertical climb, threat response*, and *appearance*. Normal *Healthy behaviors* that were not different between the groups were *shift, tail flag, lookout,* and *vocalization*. *Facedown* (lying flat on cage bottom) was not elevated in any of the groups.

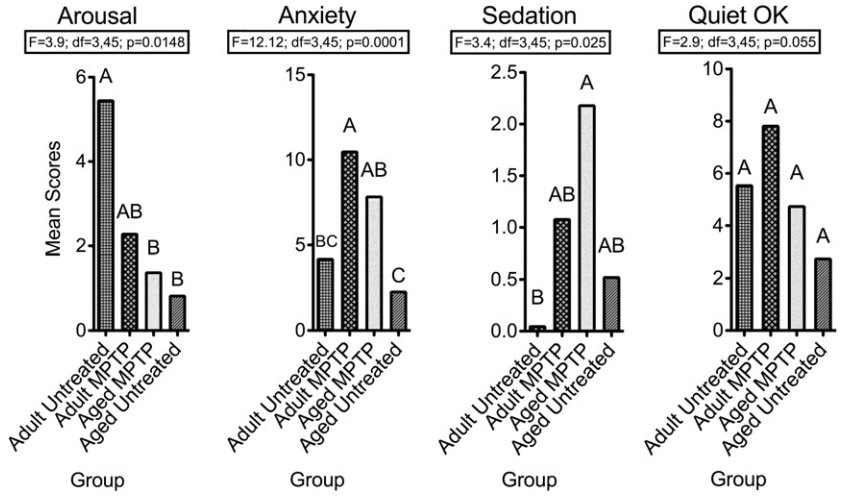

Fig. 3. Other individual factors composing normal behaviors are different between the groups. The "Aged Untreated" animals showed a decreased *arousal* compared with the "Adult Untreated" but were similar to the two MPTP exposed groups, and a decreased *anxiety* factor score as compared with MPTP-lesioned adult animals. *Sedation* and *Quiet OK* factor scores of the aged animals were similar to both the untreated adult and MPTP-lesioned adult groups. (Identical letters on the bars indicate that there were no differences between them ($p<0.05$). Different letters were statistically different. The inset boxes show the results of the ANOVAS.)

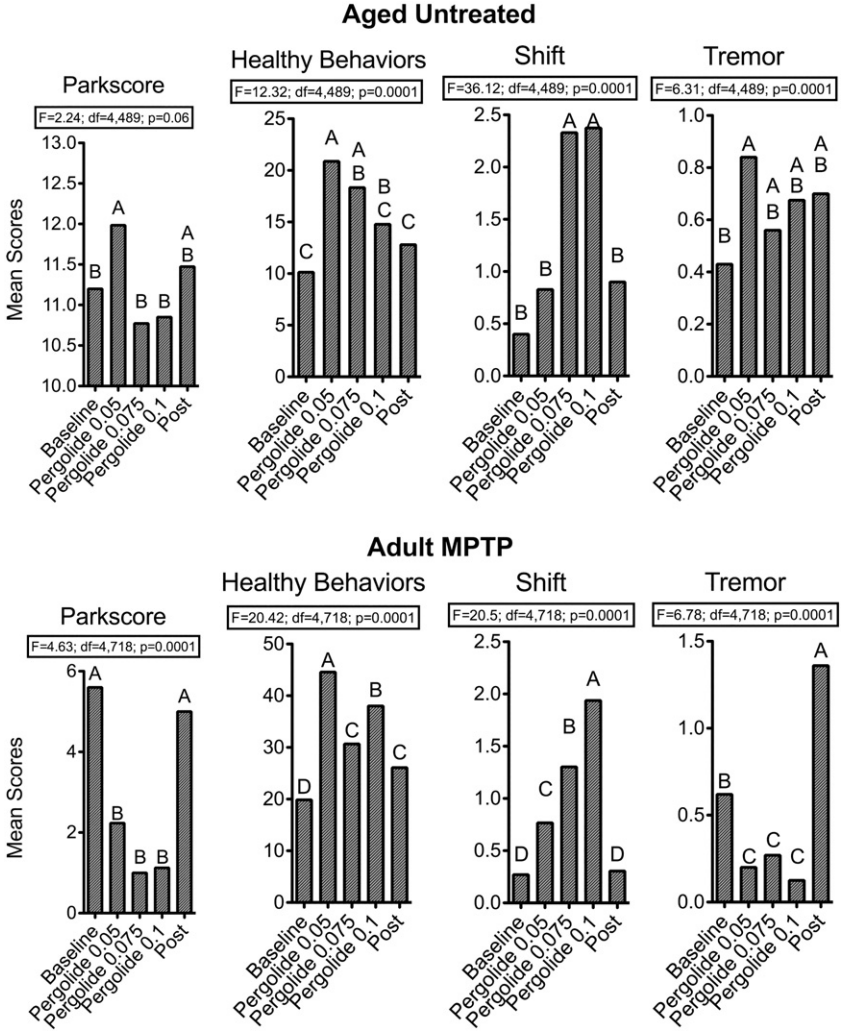

Fig. 4. Effects of Pergolide in Aged and MPTP-exposed monkeys. Low-dose pergolide treatment increased *Healthy behavior* in the aged animals, similar to increases in the "Adult MPTP" group. Improvements were not, however, seen in the *Parkscore* of the aged animals, whereas *Parkscore* was reduced in all doses in the Adult MPTP group. High-dose pergolide treatment significantly increased *shift*, which is walking, motor/ambulatory movement and *arousal* in both groups. (Identical letters on the bars indicate that there were no differences between them ($p<0.05$). Different letters were statistically different. The inset boxes show the results of the simple main effect ANOVAS.) The original multi-factor ANOVAs showed that the "Aged Untreated" group's *Parkscores* were not different overall from this "Adult MPTP" group ($p<0.29$), but their *Healthy behaviors* were different over all drug conditions ($F=7.15$, $df=1,23$, $p<0.02$).

considered the possibility that some individuals might have some process that made them more parkinsonian than individuals that were just "normally aged". The monkeys chosen for study represented all monkeys in the same age range in the colony. Seven of these aged monkeys were mild and 2 were moderate based upon the typical parkinsonian severity which follows MPTP treatment with total doses of 2.0–2.4 mg/kg administered over a 5 day period. There was no way to know how long the animals had been alive in the wild, and the actual ages of the "Aged Untreated" group were all estimated by the formula described. It was not possible, therefore, to evaluate the extent of time prior to captivity when diet and other exposures were uncontrolled, but verifiable time in captivity in the "Aged Untreated" group ranged from 19 to 24 years.

3.2. Dopamine agonist treatment

Treatment with the mixed (D-1 and D-2) dopamine receptor agonist pergolide in the "Aged Untreated" animals was shown to improve their *Healthy behavior* scores at all but the highest dose of treatment, whereas pergolide administration to the "Adult MPTP" monkeys was shown to be effective in increasing *Healthy behaviors* with two of the three doses (see Fig. 4). In contrast, pergolide injection did not significantly decrease *Parkscore* in the aged monkeys. Pergolide treatment was, however, effective in decreasing *Parkscore* in the "Adult MPTP" monkeys and increasing *Healthy behavior* components such as *shift* and other movement measures. In addition, pergolide had one opposite effect, increasing tremor in the "Aged Untreated" monkeys and decreasing it in the "Adult MPTP" group.

Administration of the preferential D2-like agonist, apomorphine and the full D1-like agonist, dihydrexidine increased the *Healthy behavior* scores of the "Aged Untreated" monkeys above baseline level, but neither they nor indirect DA agonist, L-Dopa were shown to affect the *Parkscore* of the monkeys (see Fig. 5). The effects of the agonist treatments on the component scores of the *Parkscore* were sometimes contradictory, however, as in the example of apomorphine decreasing *Poverty of Movement* but increasing *Eating Problems* in the

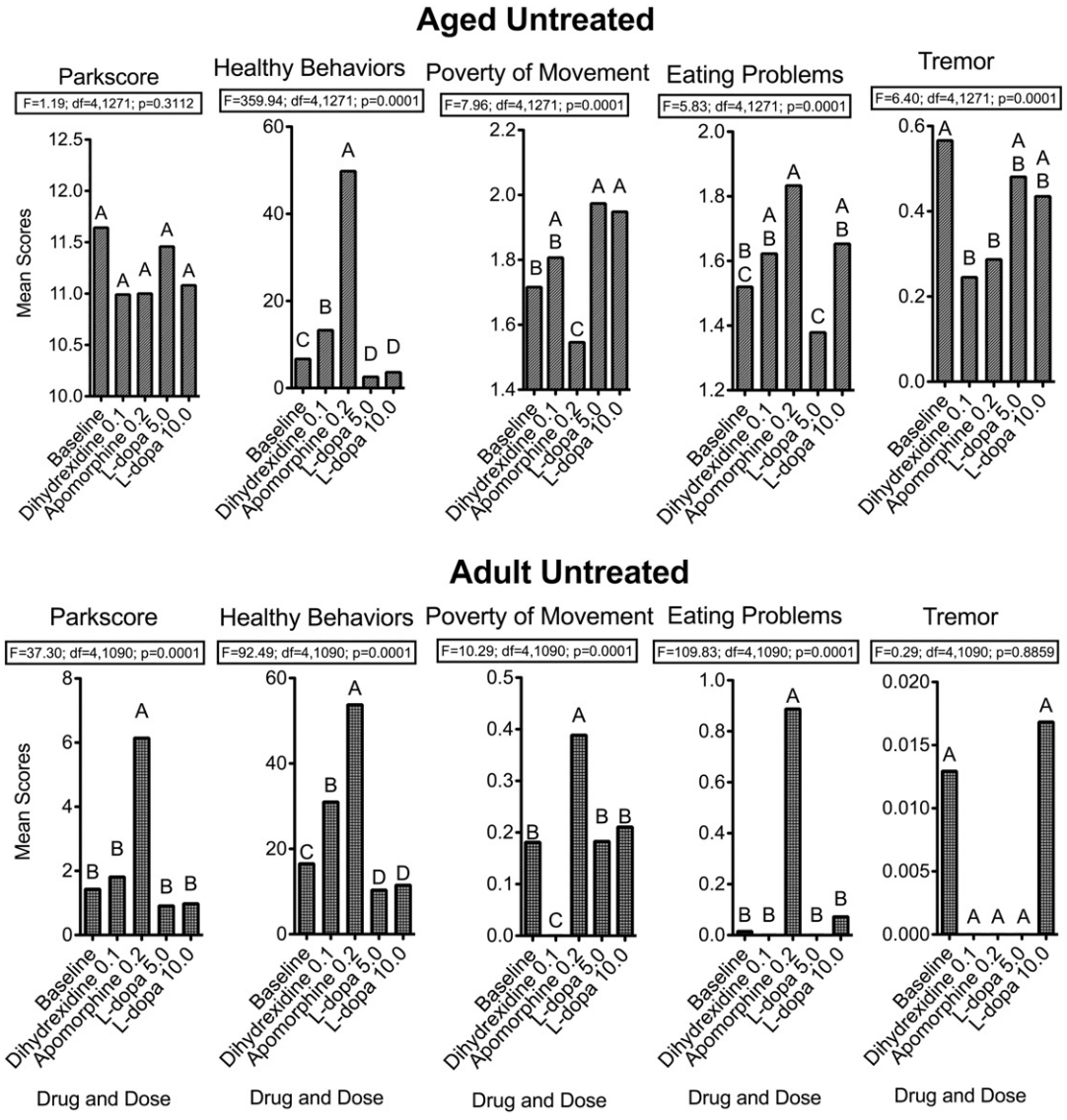

Fig. 5. DA agonists and L-Dopa treatment of Aged and Untreated Adult monkeys. *Parkscore* rating upon treatment with L-Dopa and two direct agonists was unaffected in the aged monkey group, but apomorphine significantly increased *Parkscore* in the "Adult Untreated" monkeys. Both dihydrexidine and apomorphine, but not L-Dopa, increased *Healthy behavior* in the "Aged Untreated" monkeys, and this result was mirrored in the normal adults. Apomorphine had significant effects in relation to the *Parkscore* component *Poverty of Movement* (improved) and *Eating Problems* (worsened) in the "Aged Untreated" group. In the "Adult Untreated" group, apomorphine increased *Poverty of Movement* and *Eating Problems* significantly compared with baseline. Both apomorphine and dihydrexidine decreased *tremors* in the "Aged Untreated" group compared with the baseline period, but no significant difference from the very low baseline levels was seen in the "Adult Untreated" group.

"Aged Untreated" monkeys. Both dihydrexidine and apomorphine significantly reduced *tremor* in the "Aged Untreated" monkeys. The "Adult Untreated" monkeys showed increases in *Poverty of Movement, Eating Problems*, and *Parkscore* with apomorphine.

3.3. Biochemical evaluation

Postmortem biochemical analysis of brain punch tissues demonstrated a significant deficit in dopamine levels in both the caudate and putamen for 3 aged female monkeys of similar ages to the "Aged Untreated" group, as compared to normal young adult monkey caudate and putamen dopamine concentrations (Fig. 6). In addition to dopamine concentrations being reduced, the extent of depletion was greater in the putamen (63% loss) than in the caudate nucleus (45%). This is the same as that seen in PD, in contrast to MPTP in which caudate has more DA depletion than putamen. MPTP-treated St. Kitts green monkeys with a comparable degree of parkinsonism (*Parkscore* between 5 and 12) have a greater loss of dopamine in caudate nucleus (90%) than putamen (68%) when measured more than a year after treatment (Elsworth et al., 2000). Unlike the depletions seen in dopamine concentrations, serotonin concentrations were unchanged (Fig. 7).

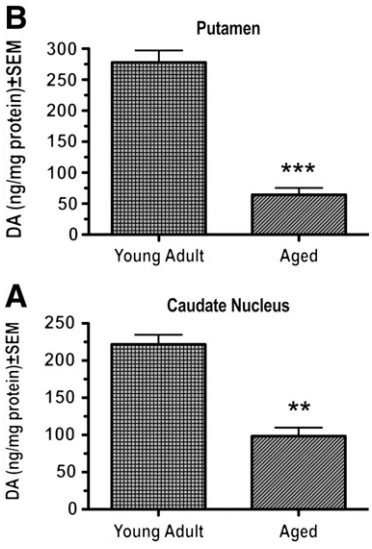

Fig. 6. Loss of dopamine in aged monkeys in caudate and putamen compared with young sex matched adults. Aged females (n = 3) showed significantly decreased dopamine levels (mean ± standard error) in either (A) caudate nucleus [unpaired two-tailed *t*-test, t(5) = 6.8, p < 0.001] or (B) putamen [unpaired two-tailed *t*-test, t(5) = 8.4, p < 0.0005] compared with young female adults (n = 4), denoted by an asterisk in the upper and lower bar graphs. A significantly greater age-dependent decrease (mean ± standard error) was observed in putamen (63 ± 3.2%) compared with caudate nucleus (45 ± 2.7%) [unpaired two-tailed *t*-test, t(4) = 4.4, p < 0.05].

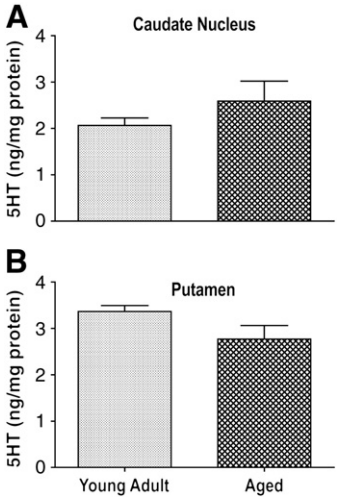

Fig. 7. No loss of striatal serotonin in aged monkeys. Young adult female monkeys (n = 4) and aged female animals (n = 3) showed no significant alteration of serotonin (5HT) levels (mean ± standard error) in either (A) caudate nucleus [unpaired two-tailed *t*-test, t(5) = 1.3] or (B) putamen [unpaired two-tailed *t*-test, t(5) = 2.1].

4. Discussion

The major finding of this study was that an untreated, aged group of female monkeys displayed significantly more classic parkinsonian signs than a sex-matched group of young adult females. These behavioral changes were similar to those of young or older adult male monkeys treated with MPTP, a neurotoxin widely used for studying the disease in monkeys. This conclusion is supported by the *Parkscore* of the animals, as well as component scores *tremor, Eating Problems, delayed initiation of movement, and Poverty of Movement*. The *arousal* factor score of the "Aged Untreated" monkeys was reduced as compared with normal sex-matched adult monkeys, replicating another change previously shown to be associated with dopaminergic cell death (Fink and Smith, 1980). Finally, biochemical analysis of three aged female monkeys post mortem confirmed the dopaminergic deficit that would be predicted by the increased *Parkscore*.

The results are complicated by gender differences between the various groups, with the primary "Aged Untreated" group being all female. These were compared with a group of young adult females, but the MPTP exposed comparison animals, both the "Adult MPTP" and the "Aged MPTP" monkeys were male. Since effects of estrogen on dopaminergic systems have been reported in St. Kitts monkeys, one of the factors in their decreased dopamine concentrations may be their post menopausal status (Leranth et al., 2000).

The finding of the same classic Parkinsonian signs in both MPTP-exposed monkeys and this group of aged monkeys raises a variety of possibilities for future exploration. Since most PD patients have presumably not acquired their condition upon exposure to neurotoxins but rather developed the condition due to multiple genetic and environmental factors as they aged, one could argue that the mild parkinsonism seen in the aged monkeys observed in this study is in some ways a more realistic model of some aspects of the disease than follows acute administration of MPTP. As a consequence of aging or chronic disease processes over 10–20 years, associated neural defects have had time to accumulate, such as alterations of post synaptic receptor numbers and sensitivity, and decrements in DA synthetic enzymes, storage, and release mechanisms (Cooper et al., 2006). All of these may be more similar to a disease that also developed over 10–20 years in the human than would be expected from an acute neurotoxin, which might have a similar phenotypic profile, but would be more likely to respond to DA replacement therapies. It should be noted that the comparison MPTP-exposed groups were only mildly affected and did not represent the full range of severity which follows the standard dose of 2 mg/kg (Redmond, in press; Taylor et al., 1994).

It is perhaps not surprising that the aged monkeys did not display L-Dopa responsiveness, mirroring the increased resistance of older PD patients to dopamine replacement and agonists (Hely et al., 2005; Newman et al., 1985). The decreased dopamine concentrations measured in some of the animals post mortem, confirms the fact that DA neurons have also been compromised or lost, and with them the capacity to convert L-Dopa to dopamine. Although in the range of human clinical doses, the dose of L-Dopa was below the 40 mg/kg threshold for improvement in hemiparkinsonism in rhesus monkeys (Kurlan et al., 1991a). The other DA agonists, apomorphine and dihydrexidine, also did not improve the overall *Parkscore*, but did have beneficial effects in some areas, such as reducing *tremor*. The

nonspecific DA agonist pergolide was the only drug successful in increasing the *Healthy behaviors*, such as "*shift*" (a measure of ambulation about the cage) in the aged monkeys. Pergolide administration did not, however, serve to decrease the overall *Parkscore*, although it apparently led to improved function and activity. This might also have reflected improvements in cognition, which were only measured indirectly in this study (Lieberman et al., 1985). If we had been measuring only cage activity or ambulation (Grondin et al., 2000), this improvement could have been interpreted as an anti-parkinson effect. However, its administration did not improve the more selective *Parkscore*.

That the aged monkeys did not fully respond to DA agonist treatment may be interpreted in different ways. It is possible that the advanced age of the monkeys rendered them less able to respond to drugs that have been successfully used to treat the symptoms of younger adult PD patients and whose beneficial effects have been replicated in a monkey model (Hely et al., 1996; Hely et al., 2005). It is also possible, but unlikely, that the behavioral effects observed in the aged moneys were entirely unrelated to dopaminergic decline, especially when specific dopamine deficits were confirmed. Mild parkinsonian symptoms in elderly humans also do not respond to standard L-Dopa treatment (Newman et al., 1985).

Since growth factors have been reported to enhance fluoridopa uptake and increasing TH immunoreactivity in aged monkeys (Kordower et al., 2000), they may be restoring and rejuvenating dopaminergic systems that are otherwise no longer able to respond effectively to the standard drug treatments that are more effective in younger patients (and monkeys). Aged mildly parkinsonian monkeys may also provide a more realistic test model for the success of cellular therapies than the young adult MPTP treated monkey. It is likely that candidates for cellular therapies would be relatively old and perhaps share some of the associated changes that may be reflected in these aged monkeys.

Another consideration is the possibility that these aged monkeys, perhaps unlike primates studied elsewhere, actually have a form of Parkinson's induced by toxic exposure to herbicides or pesticides or to some local plants that have been associated with the disease. An atypical L-Dopa unresponsive syndrome has been identified on the Caribbean island of Guadeloupe, associated with *Annona muricata* (corossol, soursop) (Caparros-Lefebvre and Elbaz, 1999; Lannuzel et al., 2006). The fruit of this plant is definitely a favorite of these monkeys, and although some exposure to it in both the wild (pre-capture) and captive population is likely, their exposures in captivity would not have reached the levels which demonstrated dopamine neurotoxicity experimentally (Lannuzel et al., 2006). Some herbicide or pesticide exposure in the pre-capture period cannot be ruled out. Two aged monkeys did show higher levels of parkinsonism than the others, but all animals in the group had significant levels, and there were no obvious factors to explain the two with the highest scores. The duration of life prior to captivity as a factor in their level of parkinsonism was impossible to determine in these wild born animals.

The decrease in dopamine concentrations in putamen between these aged monkeys and young adult monkeys (63%) is almost identical to the reductions previously reported (60%) in humans of comparable ages, although the decreases in both putamen and caudate were equivalent in the aged humans (Kish et al., 1992). The aged monkeys showed dopamine deficits that are comparable in degree to the losses observed in mildly parkinsonian monkeys exposed to MPTP, but with a pattern of greater depletion in putamen compared with caudate, which is typical of idiopathic Parkinson's disease (Hornykiewicz, 1989), without comparable reductions in serotonin.

5. Conclusion

The aged group of monkeys that was not exposed to MPTP still showed all of the main behavioral features of mild parkinsonism, even exceeding the levels of some monkeys that were exposed to MPTP in doses that are often fatal in some monkeys. This age-associated parkinsonism is atypical in that it does not respond fully to administration of dopamine replacement drugs. This failure of response raises the question as to whether these signs are due to the many other genetic, environmental, and biochemical abnormalities associated with the disease which are non-responsive to dopamine replacement (Klein and Schlossmacher, 2007). Studies of aged monkeys therefore might provide a means for identifying effective non-dopamine acting therapies. Nonetheless, comparisons of aged animals with MPTP-lesioned young adult monkeys show that the presentation of signs and deficits is almost the same. It is still unclear how aging contributes to PD, but this study shows that aged monkeys share many features of the actual disease and may be useful for modeling some aspects that may not be present after acute neurotoxin exposure.

Acknowledgments

We thank the staff of St. Kitts Biomedical Research Foundation for their dedicated care of these aged monkeys, as well as those exposed to MPTP. Leroy Whattley, O'Neal Whattley, and Clive Wilson have been caring for some of these monkeys for almost 30 years and Ricaldo Pike, DVMZ, providing veterinary care. We also thank the research assistants who did observations and carried out the drug experiments, including Ernell Nisbett, Henrietta Blake, Akinyemi Ogundana, Darnel Allen, Maurice Newton, Akeba Matthew, Xavier Morton, Samuel Phipps, Clive R Wilson, Tiffany Saunders, and Rohn Brookes.

Timothy J. Collier, Ph.D., provided helpful comments on the manuscript.

References

Adler CH, Hentz JG, Joyce JN, Beach T, Caviness JN. Motor impairment in normal aging, clinically possible Parkinson's disease, and clinically probable Parkinson's disease: longitudinal evaluation of a cohort of prospective brain donors. Parkinsonism Relat Disord 2002;9:103–10.

Adolfsson R, Gottfries CG, Roos BE, Winblad B. Post-mortem distribution of dopamine and homovanillic acid in human brain, variations related to age, and a review of the literature. J Neural Transm 1979;45:81-105.

Agid Y, Hirsch E, Anglade P, Michel P, Brugg B, Ruberg M. Aging, disease and death of nerve cells. Acta Neurol Belg 1996;96:219–23.

Anisimov SV. Cell-based therapeutic approaches for Parkinson's disease: progress and perspectives. Reviews in the Neurosciences 2009;20:347–81.

Bankiewicz KS, Oldfield EH, Chiueh CC, Doppman JL, Jacobowitz DM, Kopin IJ. Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Life Sci 1986;39:7-16.

Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 2006;38:515–7.

Bennett DA, Beckett LA, Murray AM, Shannon KM, Goetz CG, Pilgrim DM, et al. Prevalence of parkinsonian signs and associated mortality in a community population of older people. N Engl J Med 1996;334:71–6.

Bjorklund A, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res 1979;177:555–60.

Calne DB, Peppard RF. Aging of the nigrostriatal pathway in humans. Can J Neurol Sci 1987;14:424–7.

Caparros-Lefebvre D, Elbaz A. Possible relation of atypical parkinsonism in the French West Indies with consumption of tropical plants: a case-control study. Caribbean Parkinsonism Study Group. Lancet 1999;354:281–6.

Chu Y, Kordower JH. Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? Neurobiol Dis 2007;25:134–49.

Collier TJ, Lipton J, Daley BF, Palfi S, Chu Y, Sortwell C, et al. Aging-related changes in the nigrostriatal dopamine system and the response to MPTP in nonhuman primates: diminished compensatory mechanisms as a prelude to parkinsonism. Neurobiol Dis 2007;26:56–65.

Cooper JR, Bloom FE, Roth RH. The biochemical basis of neuropharmacology. New York: Oxford University Press; 2006.

Deutch AY, Elsworth JD, Goldstein M, Fuxe K, Redmond Jr DE, Sladek Jr JR, et al. Preferential vulnerability of A8 dopamine neurons in the primate to the neurotoxin 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine. Neurosci Lett 1986;68:51–6.

Elsworth J, Collier T, Redmond Jr D, Roth R, Sladek Jr J, Taylor J. Early gestational mesencephalon grafts, but not later gestational mesencephalon, cerebellum, or sham grafts, increase dopamine in caudate nucleus of MPTP-treated monkeys. Neuroscience 1996;72:477–84.

Elsworth JD, Deutch AY, Redmond Jr DE, Taylor JR, Sladek Jr JR, Roth RH. Symptomatic and asymptomatic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- treated primates: biochemical changes in striatal regions. Neuroscience 1989a;33:323–31.
Elsworth JD, Redmond Jr DE, Sladek Jr JR, Deutch AY, Collier TJ, Roth RH. Reversal of MPTP-induced parkinsonism in primates by fetal dopamine cell transplants. In: Franks AJ, Ironside JW, Mindham RHS, Smith RJ, Spokes EGS, Winlow W, editors. Function and dysfunction of the basal ganglia. New York: Manchester University Press; 1989b. p. 161–80.
Elsworth JD, Taylor JR, Sladek Jr JR, Collier TJ, Redmond Jr DE, Roth RH. Striatal dopaminergic correlates of stable parkinsonism and degree of recovery in old-world primates one year after MPTP treatment. Neuroscience 2000;95:399–408.
Emborg ME, Ma SY, Mufson EJ, Levey AI, Taylor MD, Brown WD, et al. Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. The Journal of Comparative Neurology 1998;401:253–65.
Emborg ME, Moirano J, Raschke J, Bondarenko V, Zufferey R, Peng S, et al. Response of aged parkinsonian monkeys to in vivo gene transfer of GDNF. Neurobiol Dis 2009;36:303–11.
Fahn S, Elton R, members of the UPDRS Development Committee. Unified Parkinson's disease rating scale. In: Fahn S, Marsden C, Goldstein M, Calne C, editors. Recent developments in Parkinson's diseaseNJ: Macmillan: Florham Park; 1987. p. 153–63.
Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991;114(Pt 5):2283–301.
Fink JS, Smith GP. Relationships between selective denervation of dopamine terminal fields in the anterior forebrain and behavioral responses to amphetamine and apomorphine. Brain Res 1980;201:107–27.
Fitzpatrick KM, Raschke J, Emborg ME. Cell-based therapies for Parkinson's disease: past, present, and future. Antioxid Redox Signal 2009;11:2189–208.
Grondin R, Zhang Z, Gerhardt GA, Gash DM. Dopaminergic therapy improves upper limb motor performance in aged rhesus monkeys. Ann Neurol 2000;48:250–3.
Guttman M, Fibiger HC, Jakubovic A, Calne DB. Intracarotid 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration: biochemical and behavioral observations in a primate model of hemiparkinsonism. J. of Neurochemistry 1990;54:1329–34.
Hawkes CH. Parkinson's disease and aging: same or different process? Mov Disord 2008;23:47–53.
Hely MA, Morris JG, Burns RJ, Lander CM, McLaughlin DB, Donnan GA. An Australian multicentre open label study of pergolide as an adjunct to levodopa in Parkinson's disease. J Clin Neurosci 1996;3:234–8.
Hely MA, Morris JG, Reid WG, Trafficante R. Sydney multicenter study of Parkinson's disease: non-L-Dopa-responsive problems dominate at 15 years. Mov Disord 2005;20:190–9.
Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967;17:427–42.
Hornykiewicz O. Ageing and neurotoxins as causative factors in idiopathic Parkinson's disease—a critical analysis of the neurochemical evidence. Prog Neuropsychopharmacol Biol Psychiatry 1989;13:319–28.
Irwin I, DeLanney LE, McNeill T, Chan P, Forno LS, Murphy Jr GM, et al. Aging and the nigrostriatal dopamine system: a non-human primate study. Neurodegeneration 1994;3:251–65.
Jacobowitz DM, Burns RS, Chiueh CC, Kopin IJ. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes destruction of the nigrostriatal but not the mesolimbic dopamine system in the monkey. Psychopharmacol Bull 1984;20:416–22.
Jiang C, Wan X, He Y, Pan T, Jankovic J, Le W. Age-dependent dopaminergic dysfunction in Nurr1 knockout mice. Exp Neurol 2005;191:154–62.
Kaakkola S, Teravainen H. Animal models of parkinsonism [Review]. Pharmacol Toxicol 1990;67:95-100.
Kanaan NM, Kordower JH, Collier TJ. Age-related accumulation of Marinesco bodies and lipofuscin in rhesus monkey midbrain dopamine neurons: relevance to selective neuronal vulnerability. The Journal of Comparative Neurology 2007;502:683–700.
Kanaan NM, Kordower JH, Collier TJ. Age-related changes in dopamine transporters and accumulation of 3-nitrotyrosine in rhesus monkey midbrain dopamine neurons: relevance in selective neuronal vulnerability to degeneration. The European Journal of Neuroscience 2008a;27:3205–15.
Kanaan NM, Kordower JH, Collier TJ. Age-related changes in glial cells of dopamine midbrain subregions in rhesus monkeys. Neurobiol Aging 2008b;31:937–52.
Kish SJ, Shannak K, Rajput A, Deck JH, Hornykiewicz O. Aging produces a specific pattern of striatal dopamine loss: implications for the etiology of idiopathic Parkinson's disease. J Neurochem 1992;58:642–8.
Klein C, Schlossmacher MG. Parkinson disease, 10 years after its genetic revolution: multiple clues to a complex disorder. Neurology 2007;69:2093–104.
Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y, Leventhal L, et al. Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. Science (New York, N.Y 2000;290:767–73.
Kurlan R, Kim MH, Gash DM. Oral levodopa dose-response study in MPTP-induced hemiparkinsonian monkeys: assessment with a new rating scale for monkey parkinsonism. Mov Disord 1991a;6:111–8.
Kurlan R, Kim MH, Gash DM. The time course and magnitude of spontaneous recovery of parkinsonism produced by intracarotid administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to monkeys. Annals of Neurology 1991b;29:677–9.
Lannuzel A, Hoglinger GU, Champy P, Michel PP, Hirsch EC, Ruberg M. Is atypical parkinsonism in the Caribbean caused by the consumption of Annonacae? J Neural Transm Suppl 2006:153–7.
Leranth C, Roth RH, Elsworth JD, Naftolin F, Horvath TL, Redmond Jr DE. Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implications for Parkinson's disease and memory. J Neurosci 2000;20:8604–9.
Lieberman AN, Leibowitz M, Gopinathan G, Walker R, Hiesiger E, Nelson J, et al. The use of pergolide and lisuride, two experimental dopamine agonists, in patients with advanced Parkinson disease. Am J Med Sci 1985;290:102–6.
Marsden CD. Parkinson's disease. Lancet 1990;335:948–52.
Martinez-Martin P, Gil-Nagel A, Gracia LM, Gomez JB, Martinez-Sarries J, Bermejo F. Unified Parkinson's disease rating scale characteristics and structure. The Cooperative Multicentric Group. Mov Disord 1994;9:76–83.
McCormack AL, Di Monte DA, Delfani K, Irwin I, DeLanney LE, Langston WJ, et al. Aging of the nigrostriatal system in the squirrel monkey. The Journal of Comparative Neurology 2004;471:387–95.
Mendez JS, Finn BW. Use of 6-hydroxydopamine to create lesions in catecholamine neurons in rats. J Neurosurg 1975;42:166–73.
Newman RP, LeWitt PA, Jaffe M, Calne DB, Larsen TA. Motor function in the normal aging population: treatment with levodopa. Neurology 1985;35:571–3.
Plaas M, Karis A, Innos J, Rebane E, Baekelandt V, Vaarmann A, et al. Alpha-synuclein A30P point-mutation generates age-dependent nigrostriatal deficiency in mice. J Physiol Pharmacol 2008;59:205–16.
Redmond DE, Jr. in press. Behavioral assessment in the African green monkey after MPTP administration. In: Lane EL, Dunnett, S B, editor. Animal Models of Movement Disorders: Springer, p .
Redmond Jr DE, Sladek Jr JR, Roth RH, Collier TJ, Elsworth JD, Deutch AY, et al. Fetal neuronal grafts in monkeys given methylphenyltetrahydropyridine. Lancet 1986;1:1125–7.
Redmond Jr DE, Bjugstad KB, Teng YD, Ourednik V, Ourednik J, Wakeman DR, et al. Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. Proceedings of the National Academy of Sciences of the United States of America 2007;104:12175–80.
SAS Institute I. SAS/STAT user's guide. Cary, NC: SAS Institute, Inc; 1988.
Sladek Jr JR, Redmond Jr DE, Collier TJ, Blount JP, Elsworth JD, Taylor JR, et al. Fetal dopamine neural grafts: extended reversal of methylphenyltetrahydropyridine-induced parkinsonism in monkeys. Progress in Brain Research 1988;78:497–506.
Taylor J, Elsworth J, Roth R, Sladek Jr J, Collier T, Redmond Jr D. Grafting of fetal substantia nigra to striatum reverses behavioral deficits induced by MPTP in primates: a comparison with other types of grafts as controls. Experimental Brain Research 1991;85:335–48.
Taylor JR, Elsworth JD, Sladek Jr JR, Roth RH, Redmond Jr DE. Behavioral effects of MPTP administration in the vervet monkey: a primate model of Parkinson's disease. In: Woodruff ML, Nonneman AJ, editors. Toxin-induced models of neurological disorders. New York: Plenum Press; 1994. p. 139–74.
Taylor JR, Elsworth JD, Roth RH, Sladek Jr JR, Redmond Jr DE. Severe long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the vervet monkey (*Cercopithecus aethiops sabaeus*). Neuroscience 1997;81:745–55.
Tetrud JW, Langston JW, Redmond Jr DE, Roth RH, Sladek Jr JR, Angel RW. MPTP-induced tremor in human and non-human primates. Neurology 1986;36:308.
Ungerstedt U. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In: Malmfors T, Thoenen H, editors. 6-Hydroxy dopamine. Amsterdam: Elsevier; 1971. p. 101–27.
Zaman V, Boger HA, Granholm AC, Rohrer B, Moore A, Buhusi M, et al. The nigrostriatal dopamine system of aging GFRalpha-1 heterozygous mice: neurochemistry, morphology and behavior. The European Journal of Neuroscience 2008;28:1557–68.
Zhang Z, Andersen A, Smith C, Grondin R, Gerhardt G, Gash D. Motor slowing and parkinsonian signs in aging rhesus monkeys mirror human aging. The Journals of Gerontology 2000;55:B473–80.