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Effects of Age, Gender, and Gonadectomy on Neurochemistry and Behavior in Animal Models of Parkinson's Disease

Andrea Tamás, Andrea Lubics, István Lengvári, and Dóra Reglődi

Department of Anatomy, Neurohumoral Regulations Research Group of the Hungarian Academy of Sciences, Pécs University Medical Faculty, Szigeti u 12, 7624 Pécs, Hungary

The effects of aging and gender on the neurochemistry of the dopaminergic system have been studied extensively; however, data on comparative behavioral consequences of lesions of the dopaminergic system in aging and in female animals are limited. This study presents experimental results on the behavioral and morphological outcome in young, aging, and gonadectomized male and female rats in the 6-OHDA model of Parkinson's disease. Both young and aging male animals were more susceptible to 6-OHDA than females: female rats had significantly less dopaminergic cell loss and showed a higher degree of behavioral recovery. Although the dopaminergic cell loss was only slightly more in the aging rats of the same sex, they showed more severe behavioral deficits in both gender groups. Ovariectomy did not significantly influence the dopaminergic cell loss, but behavioral recovery was worse when compared to non-ovariectomized females. In contrast, castrated males had significantly less dopaminergic cell loss than non-castrated males, but the behavioral recovery was not significantly better. The obtained results are discussed in light of the available literature on the age and gender differences in animals models of Parkinson's disease.

Key Words: Aging; gender; gonadectomy; 6-hydroxydopamine; substantia nigra; rat.

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases and is mainly associated with the progressive degeneration of dopaminergic neurons in the substantia nigra. The two most common "classical" toxin-induced PD models are the 6-OHDA and the MPTP models *(1–11)*, although other, pesticide-induced models are also used in PD research *(12)*.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a potent parkinsonian agent mainly used in monkeys and mice. MPTP is nontoxic systemically, but crosses the blood–brain barrier and it is metabolized rapidly in astrocytes by monoamine oxidase (MAO) to 2,3-dihydropyridinium, which spontaneously undergoes oxidation to 1-methyl-4-phenylpyridinium ion (MPP+). Rodents are less sensitive than primates to MPTP, mainly because of the higher level of MAO-B in the cerebrocapillary endothelium which converts MPTP to MPP+ at this site, thus preventing entry across the blood–brain barrier. MPP+ accumulates in dopaminergic neurons and selectively inhibits the mitochondrial complex I, thereby interfering with the electron-transport chain. This results in decreased levels of ATP and generation of cytotoxic oxygen radicals in the neurons *(6–9,13)*.

The selectivity of 6-OHDA is due to its accumulation by dopaminergic neurons through their high affinity uptake system. Solutions of 6-OHDA are highly unstable, and undergo fast autoxidation leading to formation of reactive radicals that results in oxidative stress–induced apoptoptic death of the dopaminergic neurons *(7,14–16)*. Accordingly, by virtue of its mechanism of action, there are close similarities between 6-OHDA and MPTP *(6)*.

One important difference between animal models and human PD is that while both 6-OHDA and MPTP rapidly destroy catecholaminergic neurons, human PD is progressive in nature *(17,18)*. While the 6-OHDA model does not mimic all pathological and clinical features of human parkinsonism, MPTP closely mimics parkinsonian features in monkeys, but its use in rodents is limited to special mice strains *(6,8)*. Another general problem is that most studies dealing with the mechanisms of neurodegeneration and/or potential therapeutic approaches use young male animals. Although young adults and even children can be affected, it is well known that PD mainly affects the elderly *(19–21)*. Age not only influences the normal functioning of the dopaminergic system, but the effect of neuroprotective agents may also be different. However, only a small percentage of studies use aging animals when testing neuroprotective strategies *(22–26)*. Also, gender differences have been shown in the prevalence and progression of the disease, but relatively few studies use female animals. Some reports point out the necessity of using aging animals in preclinical rodent models of PD in order to increase the predictive validity of the model *(27,28)*.

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Author to whom all correspondence and reprint requests should be addressed: Dora Reglődi MD, PhD, Department of Anatomy, Pecs University, Medical Faculty, Szigeti u 12, 7624 Pécs, Hungary. E-mail: dora.reglodi@aok.pte.hu

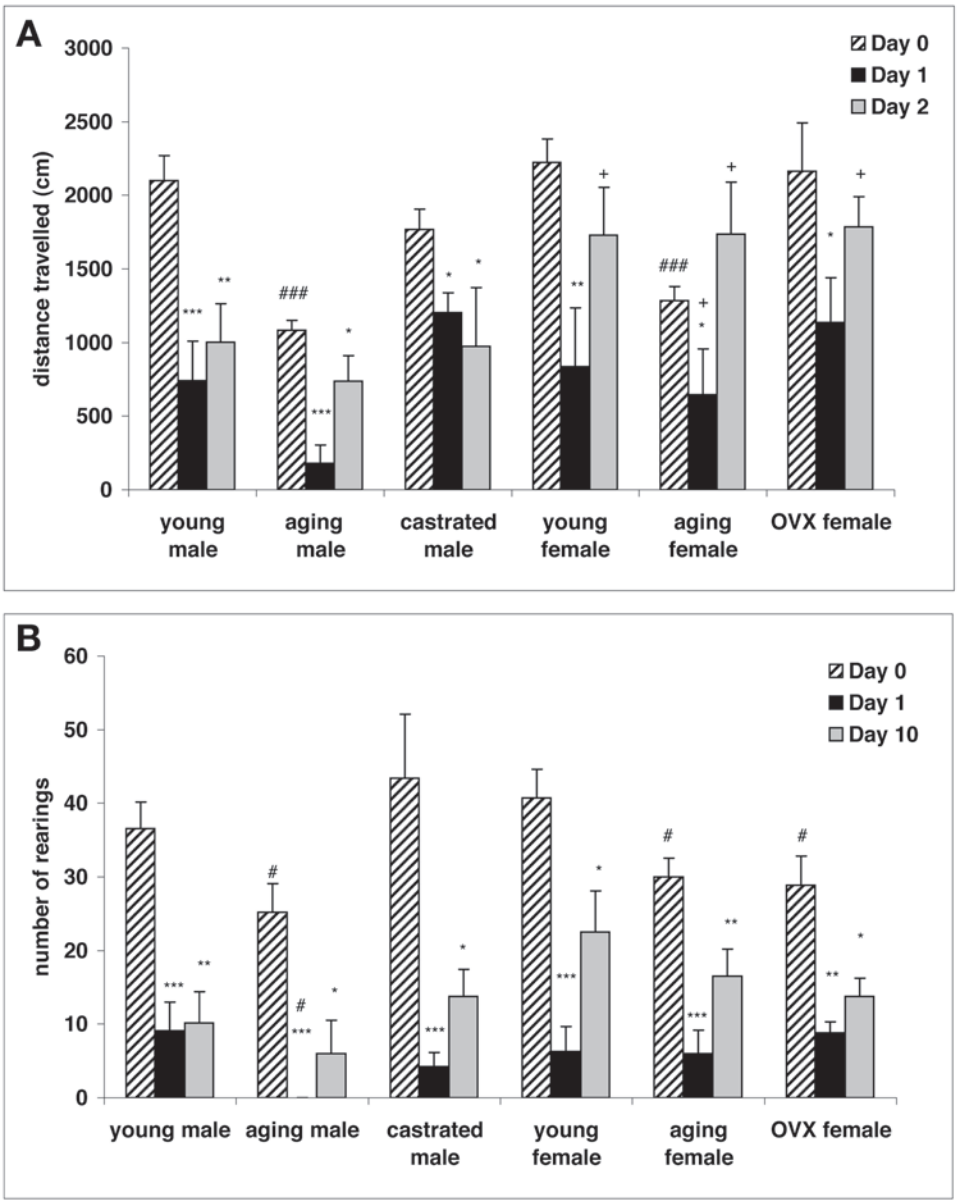

Fig. 1. Locomotor activity before (d 0) and 1 and 10 d after 6-OHDA–induced lesion of the substantia nigra. (**A**) distance covered in cm and (**B**) total number of rearings. Data are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ v 0 d values within the same group; $\#p < 0.05$, $\#\#\#p < 0.001$ vs sex-matched young group at the same time; $+p < 0.05$ vs age-matched male group at the same time.

This study presents experimental results on the behavioral and morphological outcome in young, aging, and gonadectomized male and female rats in the 6-OHDA model of PD and discusses the obtained results in light of the available literature on the age and gender differences in animal models of PD.

Results

Activity Signs in the Open Field

The distance traveled and the number of rearings were measured as signs of motor activity (Figs. 1A,B). Comparing locomotion before the operation, both aging males and females covered significantly less distance than young animals, while there was only a slight, nonsignificant difference between males and females in the age-matched groups (Fig. 1A). Gonadectomy did not influence the 0-d motor activity either, although castrated males tended to move less than non-castrated males. One day after the 6-OHDA injury, locomotion was severely reduced in all groups. Aging males showed more severe hypokinesia than young or castrated males, while there were no significant differences between the female groups. Castrated males responded with less dramatic decrease in locomotion than non-castrated males. All males were still hypokinetic 10 d later, with no differences between the groups. In the female groups, although all animals tended to move less, the difference was no longer significant 10 d later when the activity returned

to nearly normal levels. All female animals moved significantly more than their male counterparts 10 d after the injury (Fig. 1A).

Before 6-OHDA injury, both aging males and females showed less rearing activity than young animals (Fig. 1B). Males tended to rear more after castration, but the difference was not significant. Ovariectomy caused a significant decrease in rearing activity (Fig. 1B). One day after the 6-OHDA lesion, rearing activity was severely reduced in all groups, with the most striking reduction in aging males, which did not show any rearing at all. There was no significant difference between the other groups at 1 d. All animals, except for young males, showed significant recovery by 10 d, but it did not reach normal levels in any group. Although females had a pattern to rear more than their male counterparts 10 d after the lesion, it did not reach significance.

Asymmetrical Signs in the Open Field

Turning activity showed a balance between left and right turns before the operation (Fig. 2A). Severe left-biased turning asymmetry was present in all 6-OHDA-treated groups 1 d after the lesion (Fig. 2B). A similar degree of asymmetry was still observed in males 10 d after the operation with a slightly more ipsilateral turning activity, in contrast to female animals who did not show a significant difference between left and right turns (Fig. 2C).

Similarly to left–right turning activity, rearing was also balanced in all groups before the operation (Fig. 3A). One day after the injury, all groups showed significant left bias (Fig. 2B), except for the aging males, where this sign was not assessible due to the complete lack of rearings 1 d after the injury (Fig. 1B). Rearing asymmetry still persisted 10 d after the lesion in all males, including the aging male group, which showed 100% left rearing. Young females ceased to show asymmetrical rearing: there was no difference between left and right rearings. However, aging and ovariectomized females still displayed left bias in the rearing activity. The same results were obtained in active thigmotaxis (data not shown). While animals before the operation showed no side-differences in the active wall-runs, all injured rats had a significant left-bias in the wall-runs 1 d after the 6-OHDA injury. The same degree of asymmetry could be observed 10 d after the lesion in all groups, except in young females who ceased to display asymmetry.

In summary, behavioral signs show that the acute symptoms in both activity and asymmetrical measures were present in all injured animals. Females recovered significantly better by 10 d than their male counterparts. The best recovery was observed in young females, but also aging and ovariectomized females showed better recovery than males.

Histological Assessment of the Dopaminergic Neurons in the Substantia Nigra

Tyrosine hydroxylase (TH) immunohistochemistry revealed no differences between the normal, uninjured sides of the substantia nigra in the different animal groups (data not shown). There was a severe loss of dopaminergic cells in the substantia nigra pars compacta of young and aging male rats after 6-OHDA–induced lesion. The loss of dopaminergic cells on the lesioned side was more than 94% in these animals, with a slight, but not significant difference between young and aging males (Figs. 4 and 5). In contrast, castrated males had approx 60% cell loss, which was still a severe degeneration, but was significantly less than that observed in young non-castrated and aging rats. Female rats had a degree of cell loss similar to that observed in castrated males. Aging and ovariectomized females had less tyrosine hydroxylase (TH) immunopositive neurons than young females, but the differences were not significant (Figs. 4 and 5).

Discussion

The susceptibility of animals to different neurotoxins causing degeneration of the dopaminergic neurons is known to be influenced by various factors, including strain, genetic factors, endogenous variables, sex, and age *(29–36)*. Our present study shows the comparative behavioral and morphological outcome in young, aging, and gonadectomized male and female rats following 6-OHDA–induced lesion of the substantia nigra. In the sections below, our findings are discussed in light of presently available data in the same, and other, animal models of PD.

Age-Related Changes in the Neurochemistry of the Dopaminergic System

We found that although there were only slight differences in the dopaminergic cell loss between young and aging rats in both gender groups, the acute behavioral deficits and/or the degree of recovery were significantly better in the young groups.

Age-related changes in the dopaminergic system have been described that may account for the behavioral differences observed in normal and lesioned rats. Pars compacta neurons of the substantia nigra gradually degenerate over life span, and along with this, the dopaminergic functioning declines with age *(15,37–44)*. A number of studies have reported on different components of this age-related decline. Specific decreases with age in levels of dopamine and its metabolites, dopamine receptors, dopamine uptake sites, steady-state D2 receptor mRNA levels, and the dynamics of dopamine release have been shown in the brain *(45–64)*. Dopamine metabolism *(65–67)*, dopamine transporter expression, and binding of dopamine transporter ligands are reduced in aged brains *(44,68–70)*. These age-related changes in the dopaminergic system have been reviewed several times *(44,71–74)*.

Aged Animals Have Higher Susceptibility to Neurotoxins Leading to Degeneration of the Dopaminergic Neurons

Not only the normal functioning varies with age, but higher susceptibility of aged animals to neurotoxins has also been

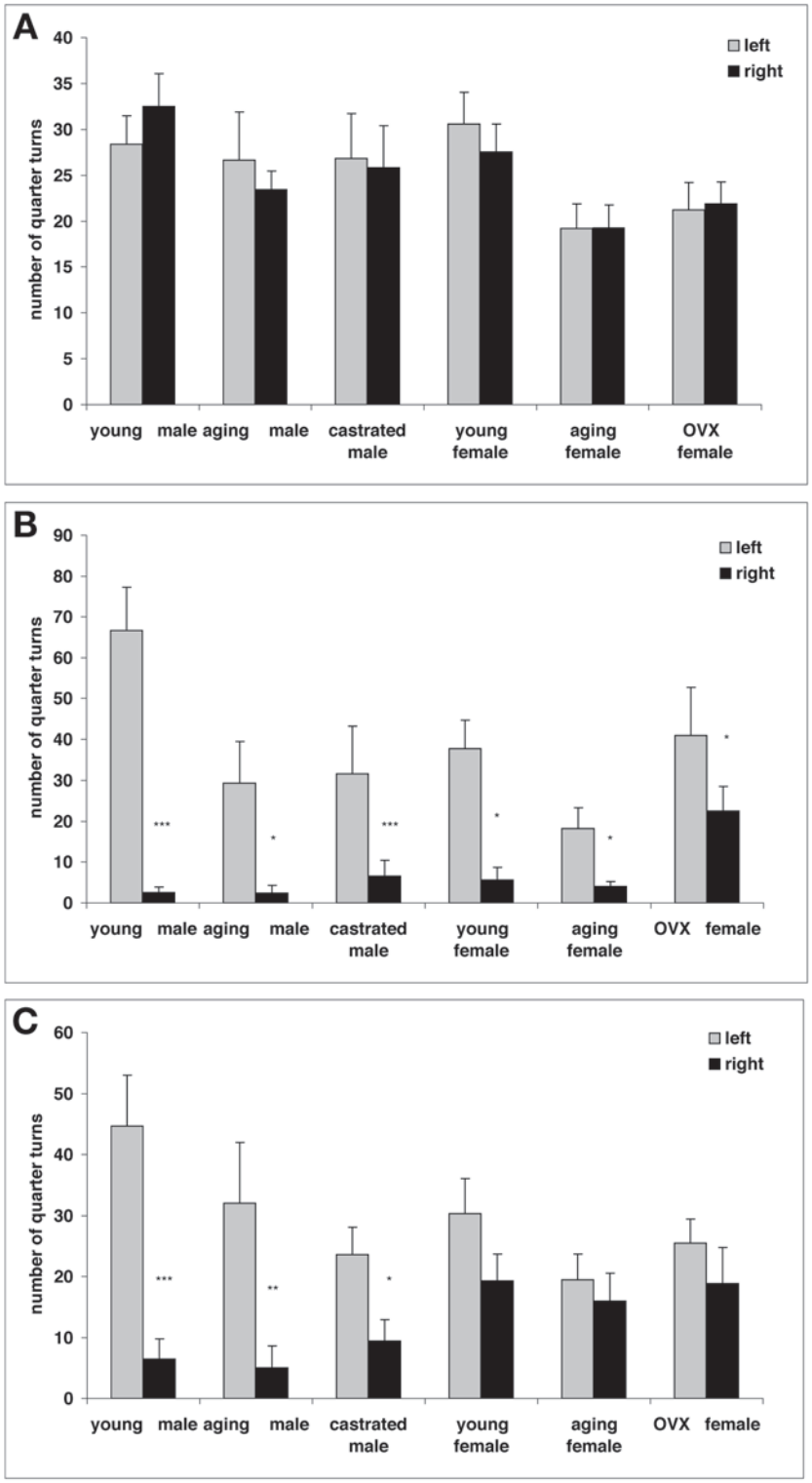

Fig. 2. Asymmetrical turning (mean number of quarter turns ± SEM) before the lesion (**A**) and 1 (**B**) and 10 d (**C**) after the lesion. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ left vs right turns.

previously described *(27,35,36,75–81)*. Aging mice show a higher degree of neuronal degeneration caused by MPTP than young mice *(27,78,80,82)*, but data on 6-OHDA are contradictory. In mice, the dopamine-depleting effect of 6-OHDA has been found not to increase with age *(83)*, while in rats, the striatal dopamine depletion after mesencephalic 6-OHDA injection is more extensive in aged that in young animals, particularly when small quantities of the toxin are given *(84)*. Intraventricularly injected 6-OHDA in aged rats also leads to a more pronounced decrease in evoked overflow of striatal dopamine and tissue levels of dopamine in the striatum and substantia nigra than in young rats

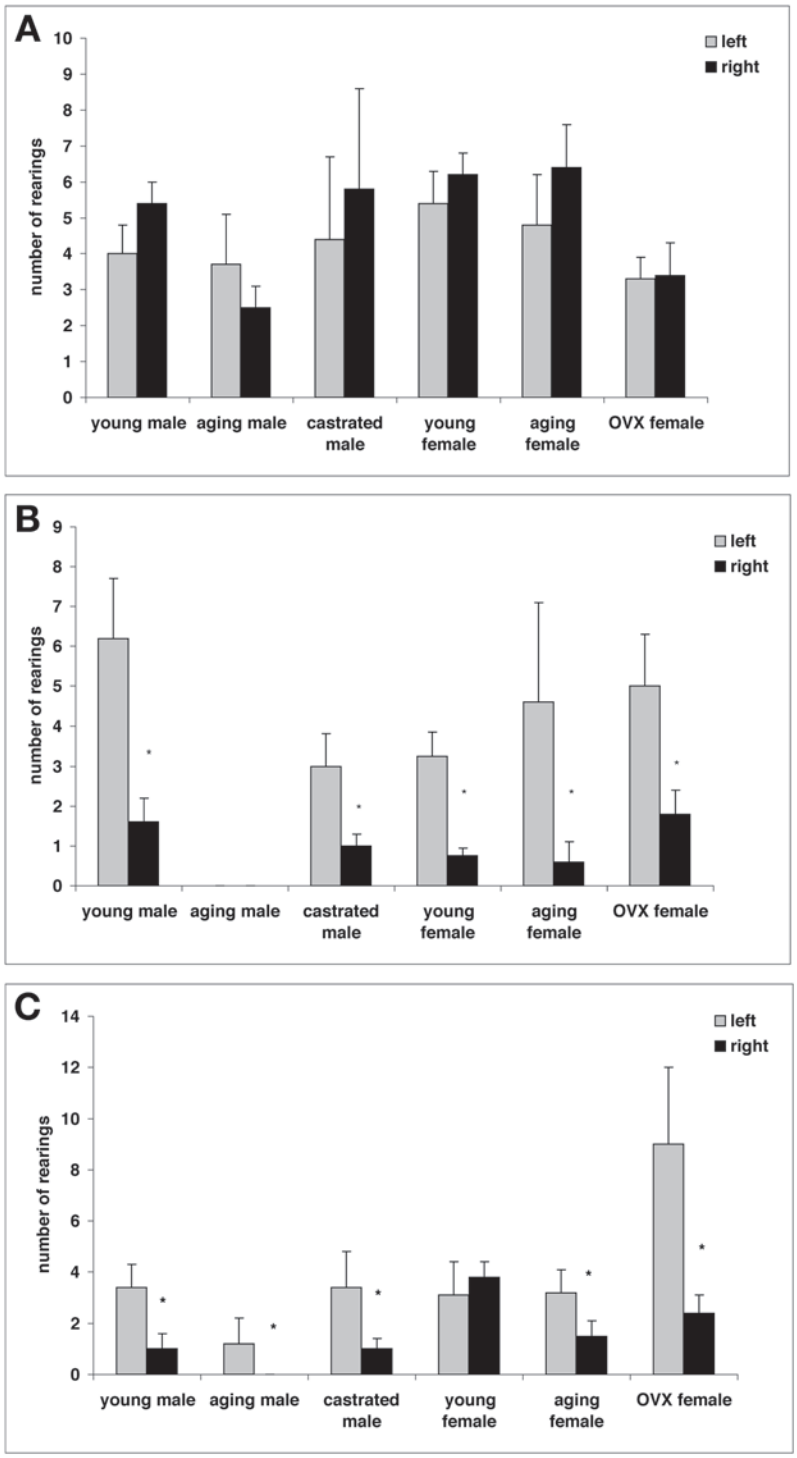

Fig. 3. Asymmetrical rearing (mean number of rearings ± SEM) before the lesion (**A**) and 1 (**B**) and 10 d (**C**) after the lesion. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ left vs right rearings.

(85). A lack of a dose dependence has been found in aged rats, which suggests that the lowest neurotoxin dose achieves a maximal behavioral effect *(84)*. Other reports also show that middle-aged and aged rats are more susceptible to 6-OHDA treatment as measured by biochemical and/or behavioral parameters *(28,85)*.

Reasons for the Higher Susceptibility of Aged Animals

The reason for the higher susceptibility of older animals is suggested to be multifactorial. Age-related changes in numerous neurochemical parameters have been proposed to be responsible for the higher incidence of PD in the elderly and the greater damage caused by neurotoxins in older ani-

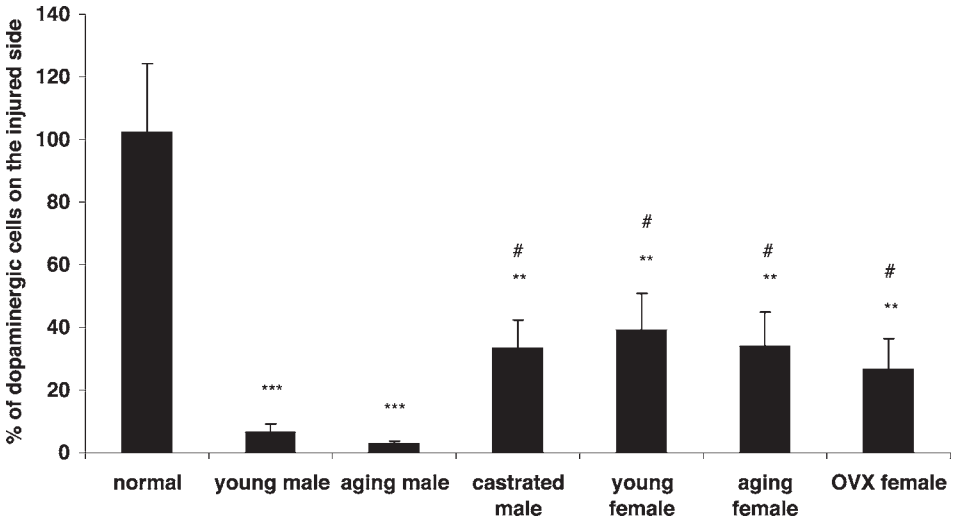

Fig. 4. The number of TH-immunopositive cells in the left substantia nigra pars compacta, expressed as mean percentage (± SEM) of the normal, uninjured side. $^{**}p < 0.01$, $^{***}p < 0.001$ vs normal rats, $\#p < 0.01$ vs young and aging male rats.

mals. Age-related increases in brain MAO and its ability to produce reactive oxygen species could contribute to neurodegeneration *(86–91)*. It has been suggested that altering kinetic factors that change the concentration of MPP+ at its target site account partly for the age-related higher susceptibility of MPTP in mice *(88,92)*.

The aged brain may be unable to produce compensatory changes in response to either age-dependent or lesion-induced neuronal degeneration, worsening lesion-induced degenerative alterations in dopaminergic neuronal morphology and function *(22–24)*. Thus, the aged brain may have a diminished capacity for neuroprotection *(93)*. Supporting this hypothesis, several protective strategies used by young brains have been reported to decrease with aging. Because oxidative stress is thought to play a major role in the degeneration of the dopaminergic neurons *(21,94–100)*, numerous studies have investigated the age-related changes in factors that lead to oxidative stress–induced damage in the substantia nigra along with impaired mitochondrial function *(101–104)*. The decrease in the antioxidant capacity, such as decreased levels of endogenous scavengers like glutathione, dehydroepiandrosterone, and melatonin have been reported *(44,105–112)*. Other important free-radical scavenging enzymes including superoxide dismutase, catalase, and glutathione reductase are decreased in the aging brain *(113)*. It has been shown by several tests that the aging brain is more sensitive to free-radical damage, like lipid peroxidation and protein oxidation *(113)*.

Several other factors may play a role in the higher susceptibility of older animals, such as age-related microglial activation in the substantia nigra in response to MPTP toxicity *(81)*, exaggerated astrocyte reactivity in the deafferentated neostriatum *(114)*, and increased accumulation of neuromelanin *(115–117)*. Elevated iron levels have also been proposed to play a role in the age-related increase of neurodegeneration and the higher susceptibility of toxins in animals models of PD *(95,96,118–121)* in addition to chronic exposure to other environmental agents, such as manganese *(21,122)*. 1-Methyl-1,2,3,4-tetrahydroisoquinoline, an endogenous monoamine that prevents the neurotoxic effects of MPTP and other endogenous toxins, shows a marked decrease in the aged brain *(123)*. Lesion-induced increases of brain-derived neurotrophic factor and glial cell line–derived neurotrophic factor are present in the young, but not in the aging nigrostriatal system *(124,125)*. More prominent c-Jun expression has been found in aging mice exposed to MPTP *(82)*. Finally, not only do endogenous protective factors decrease with age, but several reports have documented that neuroprotective strategies effective in models of PD in young animals, fail or have less effect in aged animals *(22–26)*.

Age-Related Differences in Behavioral Responses

Age-related decline in the dopaminergic system correlates with motor impairments: locomotor activity is well known to be reduced in aging animals *(39,41,63,64,126–129)*.

According to our results, the degree of dopaminergic cell loss was not significantly different between young and aging animals, but the degree of recovery and/or the acute behavioral consequences were more pronounced in aging than in young rats, similarly to the observations of Lindner et al. *(28)*. Aging males showed a more severe decline in locomotion, as shown by the severely reduced distance covered and the total lack of rearing activity 1 d after the lesion. Such differences between the acute behavioral symptoms were not observed between young and aging females. Interestingly, the recovery by 10 d was to a similar degree in aging and young males, which may be related to the nearly same degree of dopaminergic cell loss in the substantia nigra. Similar results were obtained by Marshall et al. *(84)*: aging rats showed similar degree of somatosensory recovery in spite of the more extensive acute deficits *(84)*. Also, Emerich

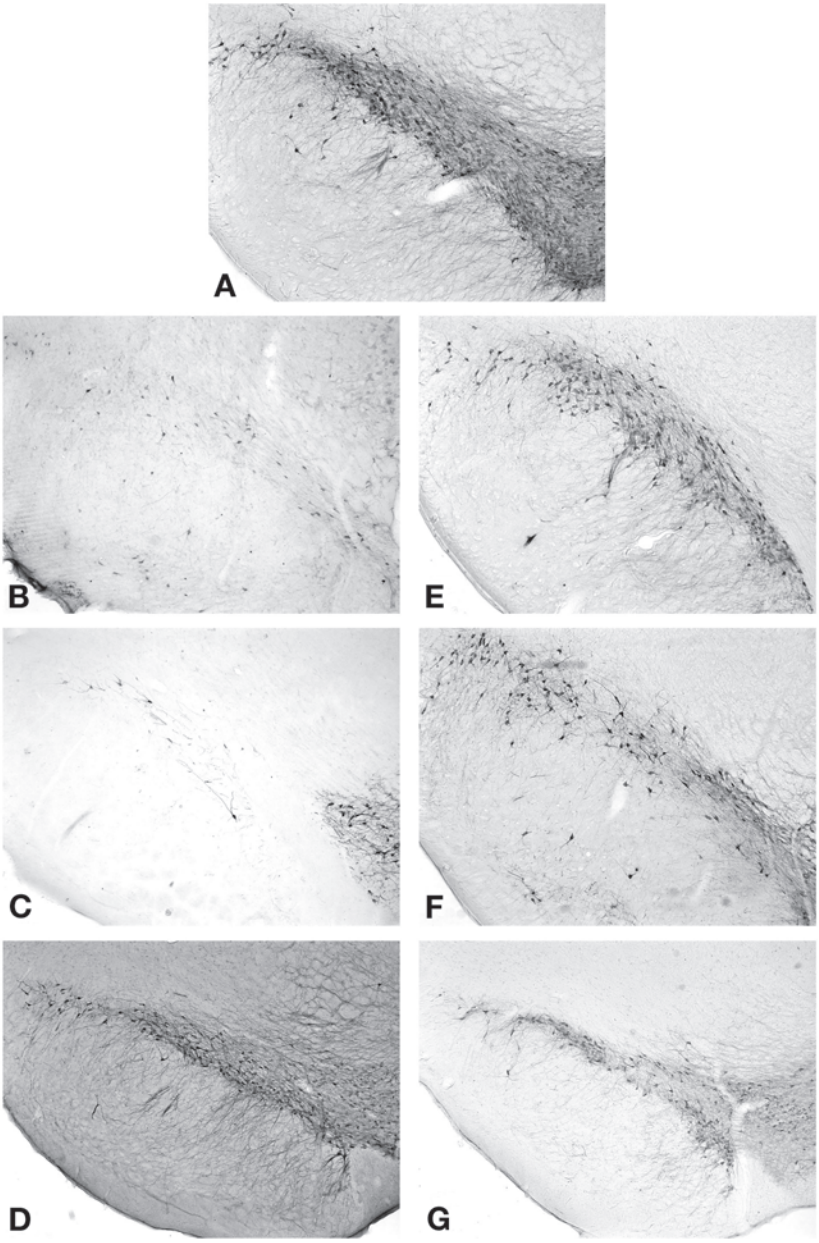

Fig. 5. TH immunoreactivity in the left substantia nigra pars compacta in a normal animal (**A**) and in a representative 6-OHDA–treated young male (**B**), aging male (**C**), castrated male (**D**), young female (**E**), aging female (**F**), and ovariectomized female (**G**) animals.

et al. *(126)* have found that the decreased locomotion of aged rats is related to neurochemical but not morphological changes in nigrostriatal dopaminergic neurons. Although most authors describe age-related loss of dopaminergic neurons in the substantia nigra *(28,39,44)*, there are several reports that found no differences in the TH-immunoreactive neurons within the mesencephalic regions between young and aging animals *(41,44,126,130)*. In accordance with these latter reports, we did not find differences in the number of dopaminergic neurons of the substantia nigra in the normal, unlesioned sides of aged and young animals. Although both aging males and females had fewer TH-positive cells on the injured side of the substantia nigra than young rats, the differences did not reach statistical significance. It is possible that the poorer behavioral response to 6-OHDA injury may be due to the slight difference in the dopaminergic cell number, but it is more probable that it is related to neurochemical, rather than morphological changes in the dopaminergic system, similarly to the findings of Emerich et al. *(126)*.

Gender Differences in the Dopaminergic System

In our present study it was found that young and aging female rats had significantly less dopaminergic cell loss than their male counterparts, which was also reflected in the better behavioral recovery following 6-OHDA–induced lesion.

Several lines of evidence suggest that gonadal steroids affect the onset and progression of neurodegenerative dis-

eases and the recovery from acute insults *(131)*. Sex differences have been shown in the pathophysiology and outcome in various neurological insults, such as ischemia, drug-induced neurotoxicity, and neurotrauma *(132–135)*. In most studies the beneficial effects of female hormones are described in different neurological insults, including PD *(133,136–142)*, but negative findings have also been presented *(134,139,143–148)*.

In spite of the contradictory data, most studies report on the beneficial effects of female gonadal hormones in animal models of PD *(149–156)*. The probable background for the gender differences found in human PD and animal models may be the well-documented biochemical and functional gender differences in the nigrostriatal pathway and the modulatory effects of estrogen *(157–168)*. Greater dopamine neuron terminal density has been found in the striatum of female rats *(169)*. Dopamine release and the maximal velocity of dopamine uptake are also greater in the striatum of female rats, independent of the estrous cycle, while no difference has been found in the affinity of the transporter for dopamine *(169)*. Estrogen modulates evoked dopamine release *(159,160,170)*, and increases dopamine concentrations by increasing TH activity and by decreasing dopamine breakdown *(148)*. Another potentially important modulatory action is represented by the estrogen-induced alterations of MAO and catechol-*O*-methyltransferase activities *(148)*. Also, estrogens increase striatal dopamine D2 receptor density and sensitivity *(148)*. However, as it has been pointed out by several authors, the effects of estrogen are not unequivocal, because many studies have reported antidopaminergic effects *(148)*.

Gender Differences in the Susceptibility to Neurotoxins of the Dopaminergic System

Not only does normal functioning of the nigrostriatal system show gender differences, but distinct responses of the nigrostriatal system have been reported to different neurotoxins. More data are available about these differences in mice than in rats. Although no gender differences have been also reported *(31)*, most studies show that female and male mice react differently to neurotoxins. In metamphetamine-induced striatal lesion, male C57BL/6J mice exhibit greater dopamine depletions in the striatum compared to females, while no such difference has been observed in BALB/c mice *(171,172)*. Similar results have been found also by others: female mice respond to both MPTP and methamphetamine with less dopamine depletions *(141)*. Pretreatment with ovarian steroids reduces MPTP toxicity *(173)*. Several other studies have reported on less susceptibility of female mice in MPTP or metamphetamine models of PD and the positive effects of estrogen treatment *(149,158,174–183)*.

Interestingly, opposite results have also been reported. In the MPTP model in mice, it has been found that high doses that led to substantial dopamine depletion in male C57 mice were lethal in females. Lower doses in the same model led to slightly more pronounced behavioral effects in female mice than in males *(184)*. Similar results have been described by Unzeta et al. *(185)*: female C57/BL mice were more susceptible to MPTP toxicity than males. In that study, the striatal dopamine depletion was similar in both sexes, but female mice did not show partial recovery in contrast to males. The higher susceptibility of female mice to MPTP in these studies could be the differences in MAO activity dependent on the estrous cycle *(185)*. Because the neurotoxic effects of MPTP depend on its conversion to MPP+ by MAO enzymes, these differences may account for the different susceptibility observed in males and females *(185)*, along with other gender differences such as hepatocyte cytochrome P450–dependent detoxication of systemically administered MPTP *(185)*. However, contradictory data have also been reported even on this issue: Miller et al. *(141)* have shown no differences in MPP+ production between female and male mice.

In 6-OHDA–induced lesions in rats, female rats respond to lower doses of 6-OHDA with significantly less cell loss than males, with less severe dopamine depletion *(132)*. Our present results confirm these findings: female rats had significantly more TH-immunopositive neurons than males, which could also be observed in aging females (>90% cell loss in young and aging males in contrast to the 60% cell loss in females). Similar degree of dopaminergic cell loss (60%) in the substantia nigra has been reported by others in 6-OHDA–lesioned female rats *(151)*.

Behavioral Difference Between Male and Female Animals in Response to Dopaminergic Lesion

In addition to the numerous studies on the gender differences in the neurochemistry of the nigrostriatal system, sex-related changes in the open-field behavior have also been previously described. Male rats are described generally as less active after the peripuberal period *(186)*. According to our present results, female rats before the operation tended to be more active in each group, but the differences did not reach statistical significance. Also, no significant differences were found in the acute behavioral symptoms: activity was reduced in males and females, and asymmetrical signs were present in all groups. However, there was a striking difference 10 d after the operation: female rats had significantly better recovery—the traveled distance reached nearly pre-injury levels, and turning asymmetry disappeared in all female groups. The best recovery was observed in young females, which also ceased to display rearing and wall-run asymmetries.

Effects of Gonadectomy in Lesions of the Dopaminergic System

Our present results show that while ovariectomy did not significantly influence the degree of dopaminergic cell loss when compared to young females, castration significantly ameliorated the morphology of the substantia nigra: the num-

ber of TH-immunopositive neurons was approximately the same in castrated males as in the female groups. In light of the influence of gonadal steroids on the susceptibility of the dopaminergic system to neurotoxins, it is not surprising that gonadectomy affects this susceptibility. However, results are not unequivocal on these effects either. Basal dopamine output has been reported to decrease after ovariectomy in rats *(187)*. It has been reported that female mice respond to metamphetamine-induced striatal lesion with less dopamine depletion than male mice, and this was not influenced by gonadectomy *(171)*. This is in accordance with our findings, that the dopaminergic cell loss was almost the same in ovariectomized and normal young rats. However, we found that castrated males also exhibited less dopaminergic cell loss than non-castrated males, while in the above-mentioned study, no such difference was found in methamphetamine-induced dopamine depletion following castration in mice *(171)*. Miller et al. *(141)* have shown that ovariectomized mice respond to MPTP toxicity with more dopamine depletion than normal mice, and that it can be counteracted with estrogen replacement, but no such effect could be observed in aged female mice *(141)*. The ameliorating effect of estrogen supplementation in gonadectomized mice has also been demonstrated by others in metamphetamine- or MPTP-induced dopamine depletion *(175, 188)*. Estrogen has also been reported to preserve striatal dopamine concentrations in gonadectomized male mice treated with MPTP, but testosterone has no such effect *(175)*. In castrated mice, evoked dopamine release is reduced when treated with testosterone in the MPTP-induced lesion, but this effect is strain-dependent *(189)*: castration in C57/B1 mice alone has failed to alter striatal dopamine concentrations in the MPTP model *(190)*. Estrogen attenuates MPTP toxicity in gonadectomized female, but not male, rats *(183)*. In rats, ovariectomy attenuates basal and induced striatal dopamine release *(159,160,191)*. In contrast, castration of male rats has been shown to have no effect on the stimulated release of dopamine from striatal tissue *(160)*. The lack of modulatory effects of testosterone also in metamphetamine-induced striatal lesions has been reported *(192)*. In 6-OHDA–lesioned rats, dopamine concentrations in the striatum of estrogen-treated females are greater than non-treated ovariectomized rats *(193)*. Another study has shown that ovariectomy enhances dopamine depletion in a 6-OHDA injury in rats, while castration in male rats reduces dopamine depletion *(132)*. In the same study, administration of testosterone to gonadectomized animals had no effect on 6-OHDA toxicity, whereas estrogen restored the extent of striatal dopamine loss *(132)*.

Although less data are available on the effects of testosterone, it has been reported that not only female gonadal hormones, but testosterone also influences the nigrostriatal physiology. Marked increases in striatal catecholamine synthesis, dopamine and metabolites and in vitro dopamine release have been shown in male rats following castration *(189)*. The majority of the data imply that testosterone may exert a tonic inhibition upon nigrostriatal dopaminergic activity *(189)*. These effects, and not only the lower levels of estrogen, can also explain the increased vulnerability of male rodents to neurotoxins of the dopaminergic system. Moreover, testosterone has been shown to alter the neurotoxic effects of MPTP in mice *(189)*, but this effect is strain-dependent, similarly to many other reports on the different neurotoxic effects of MPTP in different strains *(189)*. Our results are in accordance with these aforementioned reports: castrated male rats had significantly less dopaminergic cell loss than non-castrated males.

Behavioral Consequences of Gonadectomy in 6-OHDA–Induced Lesion

In addition to the effect of gonadectomy on the dopaminergic neurochemistry, several changes in activity have also been described. It has been shown that after gonadectomy in adult life, sex difference in the open-field activity is reduced in magnitude but persists indicating that it is not solely dependent on the presence of gonadal secretions in either sex *(186)*. Ovariectomy has been shown to reduce activity in the acute postoperative period (1–3 d) and a long-lasting reduction, starting from 3 wk after the operation in female rats *(128)*. Other reports have also shown reduced motor activity after ovariectomy, which could be reversed by estradiol replacement *(187)*. In males, castrated rats have been reported to show increased locomotor activity *(189)*.

Interestingly, gonadectomy did not significantly influence the activity of the animals before the operation in our present study. The reason for this might be the different strains of the animals, which also influences motor behavior. Also, gonadectomy did not have a striking effect on the post-injury open-field behavior, although minor effects could be observed. Ovariectomized females recovered in activity and turning asymmetry just as did young, normal females, but they did not recover in rearing and wall-run asymmetries in contrast to young, normal females. The behavioral symptoms of ovariectomized rats were similar to those of aging females. In contrast, castrated males showed behavioral signs similar to those observed in young, normal males, while aging males responded to 6-OHDA injury with more severe acute signs. Interestingly, the dopaminergic cell loss was not significantly different between castrated males and all female groups, suggesting that castration did have beneficial effects on dopaminergic cell loss, but could not protect against the behavioral deficits.

In summary, our present study showed that aging rats are more susceptible to 6-OHDA–induced lesion: although the degree of dopaminergic cell loss was only slightly more in aging animals, the degree of recovery and/or the acute behavioral consequences were more pronounced in aging rats. Females are less susceptible than males: the dopaminergic cell loss was significantly less and the behavioral recovery was significantly better than their male counterparts.

While ovariectomy did not influence the degree of dopaminergic cell loss, the degree of behavioral recovery was worse than in non-ovariectomized rats. In contrast, castrated animals responded to 6-OHDA injury with the same behavioral deficits as non-castrated rats, but the degree of cell loss was significantly less than in non-castrated males. In conclusion, there are clear age and gender differences in the susceptibility of rats to 6-OHDA–induced lesion, but the degree of behavioral outcome does not always change parallel with the degree of dopaminergic cell loss.

Materials and Methods

Animals

Wistar rats were housed under standard laboratory conditions. Animals were maintained under 12-h light/dark cycle with free access to food and water. The following animals were used: young adult males and females of 3 mo of age ($n = 10$ in both groups), aging males and females of 18–20 mo of age ($n = 8$ in both groups), and males and females of 3 mo of age that underwent gonadectomy 3 wk before the operation ($n = 8$ in both groups). All procedures were performed in accordance with the ethical guidelines approved by the University of Pécs (No: BA02/2000-31/2001).

Treatment Procedures and 6-OHDA Lesion

The dopaminergic neurons were destroyed by local administration of the neurotoxin 6-OHDA into the substantia nigra *(4,30,194)*. Rats were anesthetized with 35 mg/kg pentobarbital (Nembutal, Sanofi-Phylaxia, Hungary). All animals were given 2 μL of 6-OHDA (Sigma, Hungary) solution (dissolved in physiological saline) of 4 μg/μL concentration containing 0.2% ascorbic acid into the left substantia nigra (3 mm posterior, 2 mm left, and 8.5 mm ventral from bregma point). Injections were delivered with a Hamilton needle over a period of 5 min, and the needle was left in place for another 5 min. In castrated males and ovariectomized females, 6-OHDA treatment was performed 3 wk after surgery.

Behavioral Testing

Rats were placed in an open field of 45 × 45 cm, with 50 cm wall around. Testing was carried out under dim red light and each rat was video-recorded for 15 min at the same time of the day for each group (13–15 PM). Evaluation of the recordings was done by an investigator unaware of the treatment groups. Behavioral parameters tested were focused on two groups of signs: hypokinetic and asymmetrical signs. Locomotor activity was assessed by the distance covered in centimeters and the total number of rearings (with or without leaning against the wall) *(35,195)*. Asymmetrical behavioral signs were measured by the number of spontaneous left and right quarter-turns *(30,196,197)* and the number of left and right forelimbs use when leaning against the wall in rearing *(198)*. Active thigmotactic scanning, e.g., the number of runs along the walls (within 5 cm from the wall) of the open field with left or right side of the animals, was also recorded *(30,194,195,199,200)*. The unilateral 6-OHDA–induced lesion of the substantia nigra produces behavioral asymmetries most pronounced during the first few days after treatment, and animals may show partial recovery after a few days *(7,10,30,194,197,201–204)*. The recovery after the postoperative time is related to the degree of dopaminergic lesion: animals with severe dopaminergic depletion show no recovery after the first week, while animals with less severe lesions show partial or complete recovery. Therefore, to assess acute behavioral deficits, rats were tested 1 d after the lesion, and to assess the degree of recovery, the open-field test was repeated 10 d after the lesion. According to our previous experiments, behavioral measures at these two time-points give a good correlation with the dopaminergic cell loss in the substantia nigra *(35,205–207)*. In addition, all rats were tested 1 d before the operation in order to compare the postoperative behavioral parameters to the preoperative ones.

Histological Examination

Thirty days after the injury, rats were intracardially perfused with 4% paraformaldehyde. Serial frontal 50-μm-thick vibrotome sections from the mesencephalon were made. A mouse monoclonal antibody against TH (1: 1000, Sigma, Hungary), a marker enzyme for dopaminergic neurons, was used for immunohistochemical analysis of dopaminergic cell survival. Sections were incubated with TH antiserum for 48 h at 4°C. This was followed by incubation for 1 h in a secondary biotinylated antibody (Jackson ImmunoResearch Laboratories Inc. West Groove, PA) and in an avidin–biotinylated–peroxidase complex, following the instructions of the ABC kit (Vector Laboratories Inc. Burlingame, CA).

Digital photomicrographs were captured with a Nikon FXA photomicroscope attached to a digital camera (Spot RT Color camera). TH-immunopositive cells in each section on both contralateral and ipsilateral sides of the substantia nigra pars compacta (A9 cell group) were counted using the Scion Image computerized image analysis system, in a blind fashion. Data are expressed as percentage of TH-positive cells in the lesioned side compared to the contralateral, undamaged side.

Statistical Analyses

Results are given as mean ± SEM. Comparison between parametric data in the open field was done by ANOVA followed by Neuman-Keul's posthoc analysis. Nonparametric data in the open-field test were compared using nonparametric ANOVA test followed by Dunn's posthoc analysis. The degree of asymmetry — the number of left and right turns, rearings and runs along the walls within one group — was compared with Mann–Whitney test. The number of TH-positive cells was compared by ANOVA test followed by Dunn's posthoc analysis. Differences were considered significant at $p < 0.05$.

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