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SHORT COMMUNICATION

The impact of age and gender on the striatal astrocytes activation in murine model of Parkinson's disease

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Abstract
Objective The aim of the present study was to determine how aging and gender influence the response of astrocytes to 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) intoxication.
Materials and methods To asses the MPTP-induced astrocytes activation in nigro-striatal system, we measured the temporal changes in mRNA and protein expression of the specific astrocytic marker, glial fibrillary acidic protein (GFAP; by RT-PCR and Western blot), in the striatum of male and female C57BL/6 mice (2 and 12-month old) after 6 h and 1, 3, 7, 14 and 21 days post-intoxication.
Results We observed the increases of GFAP mRNA level post-MPTP intoxication in both young and aging males only at early time points, whereas in females (both ages) also at later time points. We noticed maximal increase of GFAP protein content on the 3rd day post-intoxication in young and aged males, whereas in females at the 7-day time point.
Conclusions The present results provide additional information of potential relevance to understand the mechanisms of gender and age-related difference in susceptibility of nigro-striatal system to MPTP insult.

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Introduction

It is known that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) generates a model of Parkinson's disease (PD) in primates and rodent by selective degeneration of mesencephalic dopamine (DA) neurons leading to the decrement of DA level in the striatum [1].

The degeneration of DA-ergic neurons is coupled with the inflammatory responses in nigro-striatal system involving astrocytes activation [2, 3]. The astrogliosis is one of the components of neurodegenerative processes in nigro-striatal system, which may be implicated in both progression and/or suppression of these processes [4–6]. Astroglia were characterized using antibodies raised against the classical intermediate filament marker, glial acidic fibrillary protein (GFAP), which plays an essential role in the formation of stable astrocytic processes in response to neuronal injury [7]. The changes in GFAP levels can be used as an index of reactive gliosis [8].

The advanced aging promotes an increase of GFAP protein content in several brain regions, and strong evidence suggests that this age-related augmentation may at least partially be followed by enhanced GFAP transcription or by slowed GFAP turnover [9]. In addition, the gender may influence production of both GFAP mRNA and GFAP protein under physiological state, as well as under some conditions of neuronal damage [10, 11]. Thus, it is reasonable to speculate that the age and gender may also influence the temporal profile of GFAP production in nigro-striatal system lesioning by MPTP administration; however, this hypothesis is still not verified.

Based on the fact that MPTP induces greater neurotoxicity in males than in females as well as in older than in young animals, and on our previous result indicating that the expression of some molecular factors of neuroinflammatory reaction, such as cytokines may be closely linked to this phenomenon [12, 13], we decided further to investigate if age and gender may influence the regulation of reactive astrogliosis in lesioned nigro-striatal system. For this purpose, we studied the temporal changes in striatal content of both GFAP mRNA and GFAP protein in young and aged female and male C57BL/6 mice following MPTP intoxication.

Materials and methods

Animals

C57BL/6 mice were housed in plastic cages under a 12 h light/12 h dark cycle and had free access to food and water. Ambient temperature was maintained at 25 ± 2°C. The following animals were used in this study: young males and females (2 months old) and aged males and females (12 months old). The experimental protocols were approved by the local ethic committee.

MPTP administration

MPTP was handled in accordance with the guidelines reported by Przedborski et al. [14]. MPTP-HCL (Sigma) was dissolved in sterile 0.9% saline. The female and male mice (both ages) received the appropriate dose of MPTP on the same day. Young females and males were injected i.p. four times with MPTP (15 mg/kg) at 1-h intervals, to the total dose of 60 mg/kg per mouse. Aged females and males were injected i.p. four times with MPTP (10 mg/kg) at 1-h intervals, the total dose per mouse being 40 mg/kg. In general, one of the most important factor for the appearance of parkinsonian signs across species is decrement of striatal DA level below the critical threshold (<80%). We previously reported (the assessment in the same time points using the same four investigated groups as in the present study) that these two selected MPTP doses produced equivalent striatal dopamine depletion (below 80%) on the 3rd day post-MPTP intoxication in young and older mice of both sexes.

Age- and gender-matched controls were injected i.p. with sterile 0.9% saline, according the paradigm, as MPTP (four times with 0.15 ml 0.9% NaCl at 1-h intervals). All injections (MPTP and 0.9% NaCl) to mice were performed between 9 a.m. and 12.15 p.m. and all mice received the same number of injections.

Animals were euthanized by cervical dislocation and decapitated at 6 h and 1, 3, 7, 14 and 21 days after MPTP intoxication and 1 day post 0.9% saline injection. The animals were decapitated in the morning (between 9 and 11 a.m.). Four to six mice were killed at each time point. The striatal samples (immediately after decapitation) were carefully dissected out from the right and left cerebral hemisphere, placed into microcentrifuge tubes, frozen on dry ice and stored at −80°C until further process. Striatum from the right hemisphere was used for the determination of GFAP protein levels and from the left hemisphere for the determination of GFAP mRNAs levels.

Additionally, the uterus was dissected and weighed from some of the female mice in both age groups to provide an index of their endocrine status.

Western blotting studies

Striata samples were homogenized, by using micropestle (Eppendorf), in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris (pH 8.0), 1% Igepal CA-630, 0.10% sodium dodecyl sulfate, 0.50% deoxycholic acid sodium salt, 0.10 mg/ml phenylmethylsulfonyl fluoride, 1.0 mM sodium orthovandate, 10 μg/ml aprotonin and 25 μg/ml pepstatin). All reagents and protease inhibitors were purchased from Sigma. After incubation on ice for 30 min, the homogenates were centrifuged at 10,000 × *g* for 20 min at 4°C. The aliquots of supernatants were taken for total protein analysis (Bradford Reagent, Sigma). Uniform protein loading was ensured by adjusting tissue samples to the same protein concentration before loading. Samples containing equal amounts of total protein (40 μg per lane) were boiled with SDS sample buffer and electrophresed on 10% SDS-polyacrylamide gels in Mini Protean II Dual Slab Cell (Bio-Rad). Proteins were electrophoretically transferred to nitrocellulose membranes using a Mini Transblot Electrophoretic Transfer Cell (Bio-Rad). Equal loading was re-confirmed by Ponceau-S staining of each Western blot lane on the membrane. The Ponceau-S was then removed by washing with distillated water. Each membrane was blocked for 1 h with 10% nonfat dry milk/0.5% Tween-20 in Tris-buffered saline. Then each nitrocellulose was incubated with a polyclonal rabbit anti-GFAP antibodies (1:1,000, Chemicon) overnight at 4°C. After three washes (for 10 min each) in TBST, the nitrocellulose filter was incubated with secondary antibodies conjugated to horseradish peroxidase (1:4,000, Amersham) for 1.5 h. The primary and secondary antibodies were diluted in TBST containing 5% nonfat dry milk. Peroxidase activity was visualized with the ECL Western blotting detection system (Amersham) according to the manufacturer's instruction. Each gel contained lanes from each time point and control brains (Fig. 1). "Density × area" measurements of each band were determined by gel analysis software program (Zero-descan, Scanalitics). Density of

investigated samples was expressed in terms of their ratio to control lanes. All investigations were performed in duplicate to assure the significance and specificity of the observations.

RT-PCR (reverse transcriptase-polymerase chain reaction)

Total RNA was isolated from brain tissue using TRI reagent (Sigma), according to the manufacturer's instructions. RNA was resuspended in 20 µl diethyl pyrocarbonate (DEPC)-treated water. The quality of RNA samples was tested by the electrophoresis of RNA on the 1.5% agarose gel containing ethidium bromide and visualization by UV illumination. The RNA was stored at −70°C until use. RNA was reverse transcribed at +42°C for 1 h with moloney murine leukemia virus (MMLV) reverse transcriptase according to the instruction of the manufacturer of the reagent (Sigma). Following the RT reaction, cDNA products were stored at –20°C until use. The resultant cDNA was amplified with gene-specific primers for.mouse GFAP: sense 5′-AGT CCC TCC GCG GCA ACG A-3′; anti-sense 5′-ACC ATC CCG CAT CTC CAC AGT CTT TAC CAC-3′and mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH): sense 5′-TGA AGG TCG GAG TCA ACG GAT TTG GT-3′ and anti-sense 5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′. Negative control reaction without template or MMLV reverse transcriptase were included in PCR amplification with primer set in parallel. As a control to eliminate variations for sample-to-sample differences in RNA extraction and conversion to cDNA, we amplified the housekeeping gene GAPDH in each sample. All subsequent PCR amplifications were performed with 20 µl reaction volumes in a Peltier Thermal Cycler PTC-200 (MJ Research, Inc.). Each primer set was optimized with regard to cycle number to insure that measurements of amplified product were obtained within the linear range of amplification. The thermal cycling parameters were as follows:

GFAP	95° C, 5 min.; 95°C, 1 min.; 55°C, 1 min.; 72°C, 1 min.; 30 cycles; and 72°C, 5 min;
GAPDH	94°C, 5 min; 94°C, 30 s; 57.5°C, 45 s; 72°C, 1 min; 30 cycles; and 72°C, 10 min.

PCR products were separated on 1.5% agarose gel stained with ethidium bromide and recorded under UV light with camera linked to an image analyzer (One-descan, Scanalitics. Inc.). All samples that were normalized to GAPDH and compared with each other were electrophoresed in the same gel. Three GFAP PCR assays per sample were performed. GAPDH mRNA, a housekeeping gene selected in this study as a control for comparison of GFAP mRNAs, was not induced by MPTP in the striatum. Additionally, we observed that GAPDH mRNA level was not altered by age and sex.

Statistical analysis

All the data were expressed as mean values ± standard deviation (SD). The arcsine transformation was used for percentage data before statistical analysis. Statistical analysis of group differences was assessed by analysis of variance (ANOVA) followed by multiple comparisons with the Fisher's protected LSD post hoc test. Differences were considered to be significant only for $P < 0.05$. All tests were calculated with the software STATISTICA PL.

Results

Uterus weights

The wet uterine weight of the 2-month mice was 101.3 ± 15.8 mg (mean + SD) and that of the 12-month-old was 253.5 ± 100.3 mg. Using the uterus weight as a general indicator of the estrogenic status, we confirmed that 12-month-old female mice had passed their peak reproductive age (as suggested by enlarged and variable uterine weights).

Levels of GFAP mRNA

GFAP mRNA was expressed at a significantly higher basal level only in aged versus young control males (Fig. 1a). In contrast, in females the aging did not alter the basal GFAP mRNA expression (Fig. 1b). While after MPTP intoxication we observed an increase in the levels of GFAP genes in all investigated group, the patterns of its expression were different in young and aged male versus young and aged female mice.

In aged male mice, GFAP mRNA was significantly elevated already at 6 h after MPTP, peaked at the 1-day time point and reduced at later time points (Fig. 1a). In young male mice, we detected significant increase in the GFAP mRNA only between 1- and 3-day time points, with maximum at 1 day post-intoxication (Fig. 1a). We also observed that the increase in the GFAP mRNA at the 1-day time-point was slightly higher in aged males compared with young males; however, these differences failed to achieve statistical significance.

In female mice (both ages), the increase in the GFAP mRNA started on the 1-day time point and remained more than the control until the 14-day time point (Fig. 1b). Moreover, we did not observe the influence of age on GFAP mRNA level between young and aged females for the duration of the assay period.

Levels of GFAP protein

Western blot analysis revealed that MPTP injection induced the increase of striatal GFAP-positive monomer at 50 kDa in all investigated groups. However, GFAP showed a somewhat different pattern of 50 kDa protein expression between male and female mice.

In young and aged males, 50 kDa GFAP level was significantly increased already at 1-day post-MPTP injection and peaked at the 3-day time point (Fig. 1d), but in aged male mice this peak was significantly more pronounced. In both males groups, 50 kDa GFAP content went down gradually during 7–21 days post-intoxication; however, 50 kDa GFAP level remained significantly more than that of the control to the end-point.

In female mice (both ages), the significant up-regulation of striatal 50 kDa GFAP started at 3 days post-intoxication (Fig. 1e). The highest increase of 50 kDa GFAP level was observed on the 7th day after MPTP lesioning in both young and aged females. In aged female mice, this increase was sustained at an unchanged level to the end-point, whereas it was reduced in young females between 14 and 21 days following intoxication (at the 21-day time point, it returned to the control level).

The immunolocalization of GFAP blotted onto nitrocellulose revealed, in addition to the normal 50-kDa GFAP, some smaller, 40–48 kDa degraded GFAP fragments in all the investigated groups (Fig. 1c). MPTP intoxication induced a marked increase in 40–48 kDa fragments of GFAP in the four experimental groups during the all assay period. However, at the 6-h time point, the mice showed monomer 50-kDA GFAP with only a very faint band at 40–48 kDa. The degraded GFAP content was the highest between 3 and 7 days post-MPTP intoxication. At later time points (between 14 and 21 days), we noticed gradual decrease of the degraded GFAP levels in all the investigated groups. That larger amount of degraded GFAP forms post-MPTP intoxication observed in the present study is in agreement with previous [9, 15] reports, suggesting that increased incomplete turnover of GFAP may be a typical physiological phenomenon in the processes of astroglial cells activation.

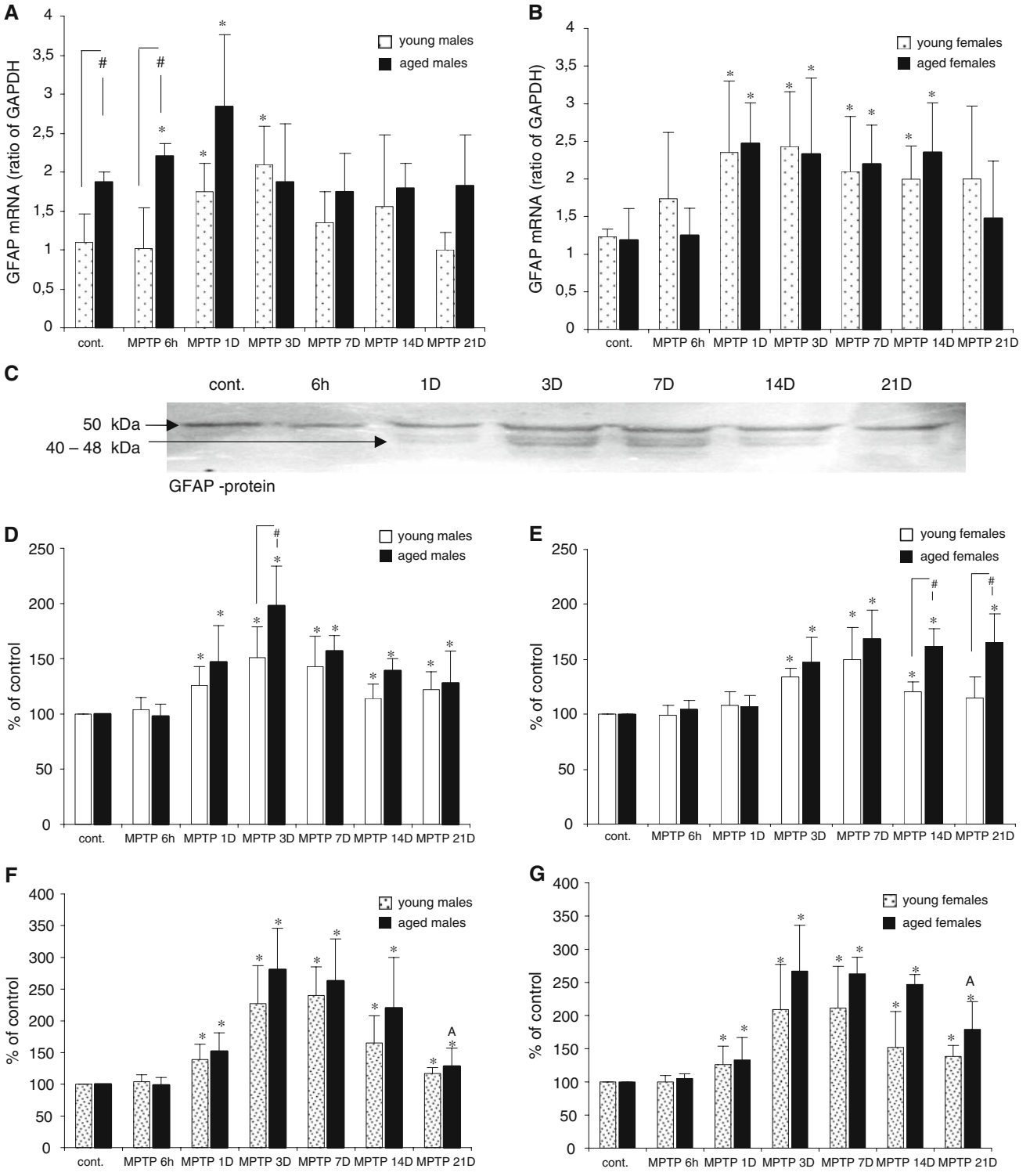
The MPTP-induced increase of 40–48 kDa GFAP fragments intensified the GFAP-immunoreactivity on immunoblots; however, the 50-kDa GFAP was clearly predominant. Between 1 and 7 days post-MPTP intoxication, we observed that the total GFAP protein concentrations were significantly increased relative to controls in all the investigated groups (Fig. 1f, g). We also demonstrated that the increase in the total GFAP protein level at the 1- and 3-day time point was the highest in aged males compared with other groups; however, this difference did not approach statistical significance. The total GFAP protein amount was gradually reduced at later time-points in all investigated groups; however, this time-dependent decrease of total GFAP protein content was much more marked in young than aged animals. Moreover, in aged animals, this total GFAP depletion was significantly more prominent in aged males compared to aged females in the 21-day time point.

Fig. 1 GFAP mRNA and protein levels in the striatum of 2 and 12 months old C57BL/6 male mice and their female counterparts in control, 6 h and 1, 3, 7, 14 and 21 days after MPTP intoxication. **a**, **b** GFAP mRNA levels were determined by RT-PCR. All samples that were normalized to GAPDH and compared with each other were electrophoresed in the same gel. Values are means ± SD ($n = 5$–6/group) of three independent experiments. **c** Representative example of striatal homogenate from aged female mouse, which was electrophresed, blotted and stained with GFAP-polyclonal antibodies. **d**, **e** Relative densities of 50 kDa GFAP. **f**, **g** Total GFAP protein. Density of investigated samples was expressed in terms of their ratio to control lanes. Ponceau-S staining of the membrane was used to monitor striatal protein loading. Values are means ± SD ($n = 4$–6/group). Results represent a typical immunoblot from two separate experiments. The statistical analysis was performed by analysis of variance (ANOVA) to determine the difference in the levels of GFAP mRNA and protein at the specific time points after lesion. Fisher's protected LSD post hoc test was used after ANOVA for statistical analysis. $^{*}P < 0.05$ indicates significant differences between control and MPTP-treated mice $^{\#}P < 0.05$ indicates significant differences between young and aged mice of the same gender. $^{A}P < 0.05$ indicates significant differences between aged males and aged females

Discussion

Our results demonstrate a few important findings. First, gender had a remarkable effect on the regulation of striatal GFAP mRNA level post-MPTP intoxication. In young and aged males, the MPTP-induced increase in the GFAP mRNA levels was observed only at early time points. In contrast, in females of both ages, this increase remained elevated also at later time points. Our findings were similar to that reported for GFAP gene expression following treatment with the neurotoxin, methamphetamine (MA) [16]. In this way, female mice that show relative neuroprotection of nigro-striatal system against MA are characterized by elevated striatal GFAP mRNA on the 7th day post-intoxication when compared with males.

It remains to be determined whether this gender dissimilarity in the GFAP response can be attributed to sex hormones, such as estrogen (Es). It is known that Es modulates astroglia proliferation and GFAP level [17–19]. Thus, we speculate that the mechanisms underlying the gender differences in striatal GFAP mRNA content post-MPTP intoxication may be related to direct Es influence on GFAP gene expression by an estrogen-response element (ERE) existing in the GFAP promoter region; for example, in the



astrocyte–neuron co-cultures, Es represses GFAP transcription [11, 20]. Furthermore, there is some evidence indicating that the regulation of GFAP mRNA may be primarily at the post-transcriptional level [21]. While it remains to be determined, however, the possibility that the degradation of GFAP mRNA may vary with gender cannot be excluded. Finally, it is interesting to note that our previous results demonstrate that following MPTP intoxication, the expression pattern of TGFβ_1 mRNA, the molecule which may influence GFAP gene activity [22, 23], is age- and gender-related and that it is similar to the pattern of GFAP mRNA, which we observed in the present study [12].

The next finding is that the aging affects the expression of striatal GFAP mRNA in lesioned striatum, but only in male mice. The earliest increase of GFAP mRNA content in striatum was observed in aged males. Interestingly, aging also promotes the augmentation of striatal GFAP mRNA in control animals, but again, only in males. While the general trend of elevated GFAP mRNA during aging in other brain areas and in both sexes of rodents is wildly recognized, there is some evidence indicating that this age-related phenomenon may demonstrate anatomically specific sexual dimorphism [24]. The fact that in aged females, we do not observe an increase in the basal GFAP mRNA expression in the striatum suggests the presence of gender-based regional variation in GFAP gene responsiveness to normal aging.

Our present results also show that the striatal GFAP (50 kDa) protein expression revealed some similarities with the time-related pattern of GFAP gene activation, indicating that the MPTP-induced intensity of GFAP immunoreactivity is mainly dependent on de novo GFAP gene expression. Interestingly, we observed the timing difference between the transcriptional activity of GFAP gene and protein expression in both the males groups. This phenomenon may be explained by some previous results indicating that GFAP is a fairly stable structural protein and it may persist at elevated levels between intervals of GFAP gene activity [22, 25]. In contrast, in females groups, we observed a more stable coincidence between GFAP gene activation and GFAP protein expression.

It is still unknown what pathological significance is implied in the gender and age-dependent up-regulation of striatal GFAP post-MPTP intoxication. Our previous results demonstrated that male mice, in particular aged males, respond faster and with greater nigro-striatal lesion to MPTP intoxication than their female counterparts [12] and that generally the nigro-striatal system of females shows significantly less neurodegeneration in response to MPTP (regardless of age) [12, 13]. These findings are consistent with previous clinical and experimental studies, which have demonstrated that PD is recognized as an age-related and sexually dimorphic disorder (with male dominance) [26, 27]. The gender differences observed in PD support the possibility that Es may play a protective role in dopaminergic degeneration. Some reports show that Es decreases striatal GFAP protein response induced by MPTP, suggesting a reduction in astrocyte responses as being coupled with estrogen neuroprotection of the nigro-striatal system [28, 29]. Because GFAP can itself directly play a role in the process of activation of astrocytes, the indicated gender-related skewing in expression of GFAP gene and protein post-MPTP intoxication in our study may have some influence on the phenotype of astrocyte response to lesion. In our opinion, the earlier astrocytes response in males may have the properties of astrogliosis with rather neurotoxic character, because this phenomenon coincides well with the greater tyrosine hydroxylase and DA depletion after MPTP in young and aged males at the early time points (reported in our previous studies [12, 13]). Furthermore, this higher constructive production of GFAP mRNA in aged male mice may make these animals more susceptible to further neurotoxic stimulation.

On the other hand, astrocytes are an important source of trophic factors and pro-inflammatory proteins, which are involved in the protection and repair of damaged dopaminergic neurons in the nigro-striatal system [3]**.** Moreover, some recent studies suggest that an interaction of estrogens with astroglial cells induce neuroprotection [30–33]. Thus, we speculate that the later astrocytes response in females may be coupled with astrogliosis involved in compensatory and survival-promoting mechanisms. It is also interesting to note that the magnitude of the striatal GFAP protein increase in response to MPTP intoxication was smallest in young female mice where the degenerative processes were slightly pronounced [12, 13, 27].

The present results provide additional information of potential relevance to understanding the mechanisms of gender and age-related difference in the susceptibility of nigro-striatal system to MPTP insult.

References

1. Bordia T, Grady SR, McIntosh JM, Quik M. Nigrostriatal damage preferentially decreases a subpopulation of alpha6beta2* nAChRs in mouse, monkey, and Parkinson's disease striatum. Mol Pharmacol. 2007;72:52–61.
2. Kurkowska-Jastrzebska I, Wrońska A, Kohutnicka M, Członkowski A, Członkowska AT. The inflammatory reaction following 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine intoxication in mouse. Exp Neurol. 1999;156:50–61.
3. Chen LW, Yung KL, Chan YS. Reactive astrocytes as potential manipulation targets in novel cell replacement therapy of Parkinson's disease. Curr Drug Targets. 2005;6:821–33.
4. Himeda T, Watanabe Y, Tounai H, Hayakawa N, Kato H, Araki T. Time-dependent alterations of co-localization of S100beta and GFAP in the MPTP-treated mice. J Neural Transm. 2006;113:1887–94.
5. Kato H, Kurosaki R, Oki C, Araki T. Arundic acid, an astrocyte-modulating agent, protects dopaminergic neurons against MPTP neurotoxicity in mice. Brain Res. 2004;1030:66–73.
6. Solano RM, Casarejos MJ, Menéndez-Cuervo J, Rodriguez-Navarro JA, García de Yébenes J, Mena MA. Glial dysfunction in parkin null mice: effects of aging. J Neurosci. 2008;28:598–611.
7. Pekny M, Pekna M. Astrocyte intermediate filaments in CNS pathologies and regeneration. J Pathol. 2004;204:428–37.
8. O'Callaghan JP, Sriram K. Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. Expert Opin Drug Saf. 2005;4:433–42.
9. Sloane JA, Hollander W, Rosene DL, Moss MB, Kemper T, Abraham CR. Astrocytic hypertrophy and altered GFAP degradation with age in subcortical white matter of the rhesus monkey. Brain Res. 2000;862:1–10.

10. Chowen JA, Busiguina S, García-Segura LM. Sexual dimorphism and sex steroid modulation of glial fibrillary acidic protein messenger RNA and immunoreactivity levels in the rat hypothalamus. Neuroscience. 1995;69:519–32.
11. Rozovsky I, Wei M, Stone DJ, Zanjani H, Anderson CP, Morgan TE, et al. Estradiol (E2) enhances neurite outgrowth by repressing glial fibrillary acidic protein expression and reorganizing laminin. Endocrinology. 2002;143:636–46.
12. Ciesielska A, Joniec I, Kurkowska-Jastrzebska I, Przybyłkowski A, Gromadzka G, Członkowska A, et al. Influence of age and gender on cytokine expression in a murine model of Parkinson's disease. Neuroimmunomodulation. 2007;14:255–65.
13. Joniec I, Ciesielska A, Kurkowska-Jastrzebska I, Przybylkowski A, Czlonkowska A, Czlonkowski A. Age- and sex- differences in the nitric oxide synthase expression and dopamine concentration in the murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Brain Research. 2009;1261: 7–19.
14. Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, et al. The parkinsonian toxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP): a technical review of its utility and safety. J Neurochem. 2001;76:1265–74.
15. Wu Y, Zhang AQ, Yew DT. Age-related changes of various markers of astrocytes in senescence-accelerated mice hippocampus. Neurochem Int. 2005;46:565–74.
16. Dluzen DE, Tweed C, Anderson LI, Laping NJ. Gender differences in methamphetamine-induced mRNA associated with neurodegeneration in the mouse nigro-striatal dopaminergic system. Neuroendocrinology. 2003;77:232–8.
17. Morale MC, Serra PA, L'episcopo F, Tirolo C, Caniglia S, Testa N, et al. Estrogen, neuroinflammation and neuroprotection in Parkinson's disease: glia dictates resistance versus vulnerability to neurodegeneration. Neuroscience. 2006;138:869–78.
18. Garcia-Ovejero D, Azcoitia I, Doncarlos LL, Melcangi RC, Garcia-Segura LM. Glia–neuron cross-talk in the neuroprotective mechanisms of sex steroid hormones. Brain Res Brain Res Rev. 2005;48:273–86.
19. Martinez FG, Hermel EE, Xavier LL, Viola GG, Riboldi J, Rasia-Filho AA, et al. Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity in the medial amygdala subnuclei across the estrous cycle and in castrated and treated female rats. Brain Res. 2006;1108:117–26.
20. Struble RG, Nathan BP, Cady C, Cheng X, McAsey M. Estradiol regulation of astroglia and apolipoprotein E: an important role in neuronal regeneration. Exp Gerontol. 2007;42:54–63.
21. Riol H, Fages C, Tardy M. Transcriptional regulation of glial fibrillary acidic protein (GFAP)-mRNA expression during postnatal development of mouse brain. J Neurosci Res. 1992;32:79–85.
22. Brenner M, Messing A. GFAP transgenic mice. Methods. 1996;10:351–64.
23. e Spohr TC, Martinez R, da Silva EF, Neto VM, Gomes FC. Neuro-glia interaction effects on GFAP gene: a novel role for transforming growth factor-beta1. Eur J Neurosci. 2002;16:2059–69.
24. Tsukahara S, Tanaka S, Ishida K, Hoshi N, Kitagawa H. Age-related change and its sex differences in histoarchitecture of the hypothalamic suprachiasmatic nucleus of F344/N rats. Exp Gerontol. 2005;40:147–55.
25. Sarthy V, Egal H. Transient induction of the glial intermediate filament protein gene in Müller cells in the mouse retina. DNA Cell Biol. 1995;14:313–20.
26. Shulman LM. Gender differences in Parkinson's disease. Gend Med. 2007;4:8–18.
27. Xu K, Xu Y, Brown-Jermyn D, Chen JF, Ascherio A, Dluzen DE, et al. Estrogen prevents neuroprotection by caffeine in the mouse 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine model of Parkinson's disease. J Neurosci. 2006;26:535–41.
28. Miller DB, Ali SF, O'Callaghan JP, Laws SC. The impact of gender and estrogen on striatal dopamine neurotoxicity. Ann N Y Acad Sci. 1998;844:153–65.
29. Tripanichkul W, Sripanichkulchai K, Finkelstein DI. Estrogen down-regulates glial activation in male mice following 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine intoxication. Brain Res. 2006;1084:28–37.
30. Dhandapani KM, Wade FM, Mahesh VB, Brann DW. Astrocyte-derived transforming growth factor-{beta} mediates the neuroprotective effects of 17{beta}-estradiol: involvement of nonclassical genomic signaling pathways. Endocrinology. 2005;146:2749–59.
31. Marchetti B, Serra PA, L'Episcopo F, Tirolo C, Caniglia S, Testa N, et al. Hormones are key actors in gene × environment interactions programming the vulnerability to Parkinson's disease: glia as a common final pathway. Ann N Y Acad Sci. 2005;1057:296–318.
32. Marchetti B, Gallo F, Farinella Z, Tirolo C, Testa N, Caniglia S, et al. Gender, neuroendocrine-immune interactions and neuron-glial plasticity: role of luteinizing hormone-releasing hormone (LHRH). Ann N Y Acad Sci. 2000;917:678–709.
33. Bains M, Cousins JC, Roberts JL. Neuroprotection by estrogen against MPP+-induced dopamine neuron death is mediated by ERalpha in primary cultures of mouse mesencephalon. Exp Neurol. 2007;204:767–76.