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Estrogen, neuroinflammation and neuroprotection in Parkinson's disease: Key role of neuron-glia crosstalk

Bianca Marchetti^{1,2}

¹OASI Institute for Research and Care on Mental Retardation and Brain Aging (IRCCS), Neuropharmacology Section, 94018 Troina, Italy; ²Department of Pharmacology, Faculty of Medicine, University of Sassari, Italy

Abstract

Post-menopausal estrogen deficiency is recognized as playing a pivotal role in the pathogenesis of a number of age-related diseases in women, such as osteoporosis, coronary heart disease and Alzheimer's disease. There are also sexual differences in the progression of diseases associated with the nigrostriatal dopaminergic (DA) system, such as Parkinson's disease (PD), a chronic progressive degenerative disorder characterized by the selective degeneration of mesencephalic dopaminergic (DA) neurons in the substantia nigra pars compacta

(SNpc). The mechanism(s) responsible for DA neuron degeneration in PD are still unknown, but oxidative stress and neuroinflammation are believed to play a pivotal role in nigrostriatal DA neuron demise. In addition, a complex interplay between genetic and environmental factors is believed to modulate the vulnerability of nigral DA neurons. Estrogen (E_2) neuroprotective effects have been widely reported in a number of neuronal cell systems including nigrostriatal DA neurons, via both genomic and non-genomic effects. Besides other mechanisms, E_2 modulation of astrocyte and microglia function in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD has recently emerged. Here we highlight E_2 as a multifunctional hormone targeting the nigrostriatal DA system during health and disease with a particular focus on gender and E_2 modulation of innate and adaptive immune responses as key factors involved in neuronal vulnerability. Special emphasis is given to the cardinal role of glia-neuron crosstalk directing neuroprotection vs neurodegeneration and the role of E_2 in switching astrocyte and microglia pro-inflammatory phenotype into a neuroprotective and anti-inflammatory state. Specifically, astrocyte and microglia response to the neurotoxin MPTP, and in particular the expression of pro-inflammatory mediators, vary according to estrogenic status with direct consequences for DA neuron survival, recovery and repair. The herein described estrogenic activation of glial anti-inflammatory and “protective” functions may provide a means to reduce the detrimental effects of neuroinflammation, while promoting cytokine activation of astroglial “pro-regenerative” functions. This mechanism might represent a compensatory/adaptive response to reduce neuronal vulnerability and/or to stimulate the repair process. These findings provide a new insight into the protective action of estrogen that may possibly contribute to the development of novel therapeutic treatment strategies for Parkinson’s disease.

Introduction

The female hormone, estrogen (E_2), plays a fundamental role in a multitude of physiological processes in mammals, including reproduction, cardiovascular health, skeletal growth and bone homeostasis, immune and cognitive functions. One key aspect is represented by the critical action of E_2 in dictating gender-specific processes of brain development, differentiation and activity [1,2]. Also within the immune system E_2 chiefly contributes to the generation of the sexually driven immunological dimorphisms [3] and plays a pivotal role in the bidirectional crosstalk between the neuroendocrine and immune systems [4]. Within the central nervous system (CNS), E_2 promotes neuron growth, prevents neuronal cell atrophy, and regulates synaptic plasticity [1]. Besides other neuronal systems, estrogen is a well recognized physiological regulator of nigrostriatal dopaminergic (DA) neurons, during

development, in adulthood and during neuronal degenerative processes, such as those involved in Parkinson's disease (PD), a progressive degenerative disorder characterized by the selective loss of mesencephalic DA neurons in the substantia nigra pars compacta (SNpc) [5].

The loss of dopaminergic afferents to the striatum and putamen results in extrapyramidal motor dysfunction, including tremor, rigidity, and bradykinesia [6]. The mechanism(s) responsible for DA neuron degeneration in PD are still unknown, but oxidative stress, excitotoxicity, depletion of endogenous antioxidants, reduced expression of trophic factors and dysfunction of the protein degradation system are believed to participate in the cascade of events leading to DA neuron death [6-10]. Recent evidence clearly indicates that neuroinflammatory mechanisms may play an important role in the pathogenesis of PD and inhibition of inflammation proposed as a promising pharmacological strategy [11-25]. Therapeutic interventions generally consist of the management of motor symptoms with either L-DOPA or dopaminergic agents, but they don't delay progression [6]. In man, epidemiological evidence suggests that chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) reduces risk by about 45% [19,20].

In this paper, after a brief summary of gene-environment interactions and hormonal interplay in PD vulnerability, the experimental and clinical evidence documenting the vital role of estrogen as a survival, neurotrophic and neuroprotective factor for the nigrostriatal DA system will be reviewed. The importance of both peripheral and central estrogen synthesis and action via estrogen receptors, as well as the impact of estrogen deficiency as a risk factor for vulnerability to PD is then summarized. Neuroinflammation will then be introduced as a critical hallmark of nigrostriatal DA degeneration and estrogen's influence on both innate and adaptive immune mechanisms outside and within the brain will be discussed. The cardinal role of estrogen in the MPTP model of PD will be introduced together with our own studies documenting a key role for E₂-induced modulation of the astroglial cell compartment in nigrostriatal DA neuron protection against oxidative and nitrosative stress cascades engendered by MPTP [25].

Complex gene-environment interactions play a crucial role in increasing vulnerability to Parkinson's disease: A pivotal role for hormones of the stress and gonadal axes

Although several genes that cause certain forms of inherited PD have been identified, most cases of PD appear to be sporadic and likely represent an interplay between both genetic and environmental influences [26]. Polymorphisms

in candidate genes involved in dopamine metabolism, mitochondrial function, lipoprotein metabolism and xenobiotic detoxification have been described [26]. In addition, polymorphisms of inflammatory genes have been also reported, including inducible nitric oxide synthase iNOS [27], IL-6, and estrogen receptor beta (ER- β) gene [28]. In addition, smoking and pesticides affect the probability of developing PD [26,29]. Rural living, dietary factors, exposure to metals, head injury, and exposure to infectious diseases during childhood have also been suggested to increase risk [23,26,29].

Of particular mention, genetic factors may interact with early life events such as exposure to hormones, endotoxins or neurotoxins, thereby influencing disease predisposition and/or severity (Fig. 1). In addition, developmental exposure to environmental toxins (such as pesticides/herbicides) in concert with other environmental (i.e. endotoxins; hormonal dysfunctions) and/or genetic “predisposing” factors, may synergistically increase dopaminergic neuron vulnerability [22-31].

In particular, two key axes, which are responsible on the one hand for the stress response and modulation of inflammation, and on the other, for reproductive hormone homeostasis, are involved: the hypothalamic-pituitary-adrenocortical axis (HPA) and the hypothalamic-pituitary-gonadal (HPG) axis [4,22,-25]. The stress hormones glucocorticoids (GCs), the most potent anti-inflammatory and immunosuppressive agents known, via their cognate receptors (GRs), represent crucial vulnerability factors in experimentally-induced Parkinsonism [22,24,31] via critical neuroendocrine-immune interactions [31,32] are of particular interest. Hence, early embryonic life exposure to GR antisense RNA in transgenic mice, leading to an abnormal response to stressful, inflammatory and immune stimuli [33-36], dramatically increases DA neuron vulnerability to MPTP, via an exacerbation of the neuroinflammatory reaction [22,24,31,36,37].

Conversely, environmental enrichment can confer resistance to MPTP [38], thereby underlying a crucial role for the environment and the HPA axis, which is highly sensitive to environmental manipulations [39,40], as critical factors involved in nigral DA neurons preservation.

We have proposed that an altered dialogue between the neuroendocrine and the immune systems via the HPA axis, during development, may irreversibly shape glial cells and «program» long-term effects in the mechanisms regulating immune responsiveness to inflammation, thereby contributing to individual vulnerability, propensity and predisposition to inflammatory, autoimmune and neurodegenerative disorders [22-25,31,36,37] (Fig. 1). Developmental bidirectional interactions between the reproductive and the immune systems during peri-natal life depend upon gender and are known to dramatically impact on the maturation and function of neuroendocrine and immune systems, with potential influences for predisposition

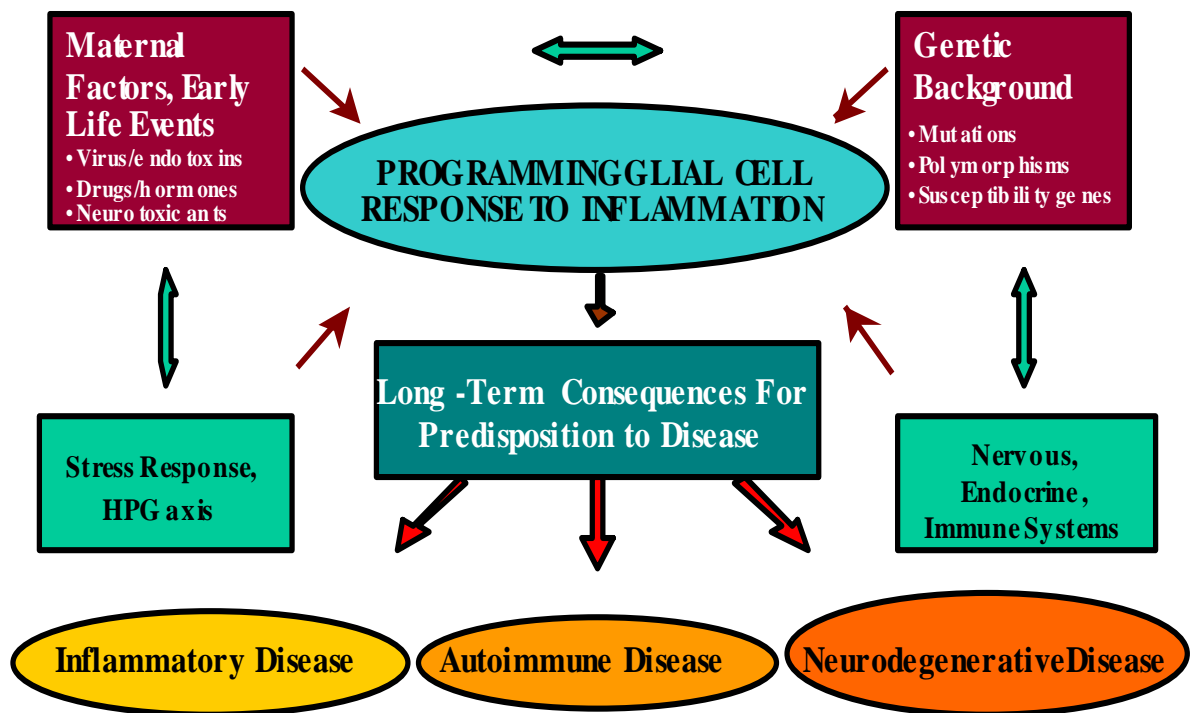


Figure 1. Schematic representation of the impact of perinatal genetic, hormonal and environmental interactions on inflammatory glial cell response and individual resistance or susceptibility to inflammatory diseases during adult life. Genetic factors (e.g., sex, gene mutations, polymorphisms/ susceptibility genes) can interact with maternal hormonal factors and external agents to which mother and fetus are exposed (drug treatments, bacteria, viruses, endotoxins, and/or environmental toxins such as heavy metals or pesticides), to alter the development of the neuroendocrine-immune system, in particular the hypothalamic-pituitary-adrenocortical axis (HPA) and the hypothalamic-pituitary-gonadal (HPG) axis. The pivotal target of the overall interactions is glia, a key component of the neuroendocrine-immune system. Thus, an altered dialogue between the neuroendocrine and the immune system during development may irreversibly shape glial cells and «program» long-term effects in the mechanisms regulating immune responsiveness to inflammation, thereby contributing to individual vulnerability, propensity and predisposition to inflammatory, autoimmune and neuromental disorders [for details see text]. Within this context, a key component is represented by the interactions between the neuroendocrine and immune systems during development and the response of the astroglial cell compartment during adult life.

and/or increased vulnerability to various disease entities in adult life, particularly noteworthy are the mutual interactions between the HPA and HGA axes [4,41-44].

Importantly, gender and sex steroid background also appear to strongly modulate vulnerability to PD, with mechanisms not completely elucidated. We have recently emphasized E_2 modulation of glial neuroinflammatory reaction in MPTP-induced experimental Parkinsonism [22,25]. In particular, by assessing temporal correlations between the astroglial cell response to MPTP,

endogenous E₂ and changes in DA neuron functionality we documented a significant contribution of the astroglial cell compartment, and neuron-astrocyte-microglial crosstalk in E₂-induced neuroprotection [22,25]. In the following sections the unique ability of estrogen to exquisitely modulate the nigrostriatal DA system in health and disease will be emphasized.

Estrogen is a multifunctional hormone targeting the nigrostriatal dopaminergic system: Menopause and E₂ deficiency as risk factors for nigrostriatal DA neuron demise

Female animals are protected from many forms of neurological insults and degeneration relative to their male counterpart, whereas loss of endogenous estrogens through pharmacological inhibition, surgical ovariectomy, or reproductive senescence eliminates this benefit. Likewise, post-menopausal estrogen deficiency is recognized as playing a pivotal role in the pathogenesis of a number of age-related diseases in women, such as osteoporosis, coronary heart disease and certain neurological diseases, including PD. The extensively investigated process of normal reproductive decline in women and experimental animals, known as reproductive senescence, involves three major players : the ovary, the brain and the immune system, and the marked alterations in the interactions of these crucial homeostatic systems are associated with the increased vulnerability of female gender to a number of aged-associated diseases [4,45-47]. Menopause, a permanent cessation of female cyclicity in primates, is characterized by the total exhaustion of ovarian follicles and is also accompanied by a virtual disappearance of E₂ from the circulation. A rapid and marked drop in estrogen levels that prevails for the rest of life has significant health implications for women, namely increasing their risk of obesity, cardiovascular diseases, osteoporosis, cancer and apparent psychological disturbances. Over the past decade the impact of the prolonged post-menopausal hypoestrogenic state on the CNS of women has been increasingly appreciated. The endogenous ovarian steroid 17β estradiol, a potent neurotrophic and neuroprotective factor is a prime modulator of the aging process in the brain [45-47]. A substantial body of evidence clearly indicates that the increased vulnerability of aging women to brain injury may be causally related to their reduced blood E₂ levels [45-47].

Regarding the nigrostriatal system, a number of epidemiological studies have reported that the incidence and prevalence of PD is higher in men than in women [48,49]. Post-menopausal E₂ deficiency has been reported to cause a worsening of Parkinson-related symptoms, whereas the severity of symptoms in women with early PD is diminished by the use of E₂ [50]. Association

between E₂ receptor gene polymorphism and PD disease with dementia and with age of onset of PD [28], have been reported. These clinical results are supported by a body of experimental evidence indicating that the nigrostriatal DA system is subject to modulation by E₂ in rodents and non human primates [5,51]. Thus, the nigrostriatal DA system is exquisitely sensitive to gonadal hormone influence and sexual differences are present in several parameters of the nigrostriatal DA neurons, as well as in the progression of diseases associated with this system [5,51-64]. Indeed, E₂ has been defined as a neuroprotectant for the nigrostriatal DA system [51]. The neuroprotective effects of E₂ have been reported against DA neurotoxicity induced by 6-hydroxidopamine (6-OHDA), metamphetamine, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD [58-64].

Collectively, the overall summarized results, coupled to a wide variety of other findings [64] convincingly document that the nigrostriatal DA system is a preferential target of E₂, and loss of peripheral E₂ at menopause may represent a crucial risk factor for PD pathogenesis.

Local brain estrogen synthesis and aromatase activity in neuroprotection: Aromatase down-regulation as a critical pre-disposing factor in neurodegeneration

Estrogen is synthesized in a number of human tissues. Aromatase, the enzyme in steroidogenesis which is responsible for the conversion of testosterone and other C19 steroids to estradiol, has been detected in the CNS in a wide variety of vertebrate species [65]. In the brain E₂ is formed locally in neural tissue from the conversion of precursor androgens by aromatase, and estrogen may act in a paracrine/intracrine fashion [66,67]. There has been a longlasting debate as to whether this enzyme is expressed in glial cells and/or neurons. It is now widely accepted that aromatase is expressed both in glial cells and neurons as shown in the mRNA and protein levels [68,69]. While under normal circumstances the expression of aromatase in the CNS of mammals appears restricted to neurons, the expression of the enzyme in astrocytes is induced by a number of stimuli [69]. One important consideration is that different types of brain injury induce *in vivo* the expression of aromatase in reactive astrocytes; furthermore the expression of aromatase by reactive astrocytes is neuroprotective, because the pharmacological inhibition of the enzyme in the brain exacerbates neuronal death after different forms of mild neurodegenerative stimuli [69].

Further experimental and clinical evidence indicate that brain aromatase activity may be neuroprotective. Indeed, a neuroprotective effect of estrogen and aromatase have been demonstrated with respect to stroke, Alzheimer's

disease and epilepsy [69]. In the brains of female AD patients, total and free E_2 levels are low indicating that local E_2 synthesis is impaired in AD brain [70]. In addition a significant negative correlation was found between aromatase mRNA levels and amyloid plaque density in AD brains [70]. In addition, aromatase gene knock out (ArKO) mice showed enhanced hippocampal neuron loss in response to neurotoxin compared with Wt mice [70].

Concerning the nigrostriatal DA system, during the perinatal period of rat brain development aromatase is transiently expressed from embryonic day 17 (E17) until post-natal day 10 (P10) in the ventral mesencephalon [71]. In contrast, aromatase activity in the striatum is detectable before birth at low levels but increases post-natally and persists in adulthood [71,72]. These data are also in accordance with the presence of ERs in these specific brain regions [73]. Thus, the capacity of estrogen formation is present at distinct phases of mid brain development indicating the ability of mesencephalic cells to synthesize estrogens perinatally. Because considerable E_2 production can be found within the embryonic midbrain, this implies that E_2 intrinsically synthesized rather than systemic estrogen is the source for the observed estrogen developmental effects [64]. Hence, E_2 functions as a trophic factor helping in the establishment of and stabilization of early DA connections and to initiate functional dopamine transmission [64]. Indeed, the developmental expression of tyrosine hydroxylase (TH), the rate limiting step in dopamine biosynthesis coincides with transient aromatase expression in the midbrain [74], whereas blocking “in utero” aromatase activity with an aromatase inhibitor caused a robust inhibition of TH mRNA and protein levels [74]. In addition, using cultured midbrain cells, the ability of E_2 to increase TH mRNA and protein levels was further documented, an effect reversed by the application of the ER antagonist ICI 182,780 [74]. In adult life, however, E_2 formation declines within these areas and peripheral estrogen reaching the brain via peripheral circulation takes over this function [64]. With regards to other DA neuronal systems, aromatase activity has been shown to be involved in maintaining the functional integrity and survival of hypothalamic DA neurons. Hence, in one year-old male ArKO mice, the absence of E_2 promotes apoptosis in the DA neurons of the arcuate nucleus of the hypothalamus and the medial preoptic nucleus, which in turn has important implications for the regulation of energy balance and behavioural aspects [75].

Together these data document that brain aromatase activity may be critical during ontogenic development of the nigrostriatal DA system, whereas in adult life circulating E_2 influence morphology and functional activity of these neurons. On the other hand, given the potent neurotrophic and neuroprotective effect of E_2 and aromatase expression in reactive astrocytes, the consecutive increase in the local production of estradiol in the brain at injured sites, might further represent an endogenous paracrine/intracrine means to reduce the

extent of neurodegenerative damage [69]. Recent genetic studies also indicate that the brain aromatase gene may modify the risk of several diseases, including neurological diseases [76].

Collectively, the overall findings indicate, therefore, that besides the age-dependent peripheral E₂ deficiency summarized in the previous section, down-regulation of brain aromatase activity as a result of ageing, genetic defect or pharmacological inhibition, may affect predisposition to neurodegenerative processes, including PD.

Multiple genomic and non-genomic mechanisms(s) are responsible for E₂-induced neuroprotection

The mechanism(s) by which E₂ protect neurons is currently under intense investigation and involve receptor- and non receptor-mediated effects. Both subtypes, ER- α and ER- β , mediate the effect of E₂ in the brain. Both ERs belong to the steroid nuclear receptor superfamily, members of which share a common structural architecture [77]. Even though the two types of ERs coexist in different brain regions, ER- α and ER- β are also found independently in other regions [78].

ER- α appears predominantly expressed in classical estrogen receptor target tissues such as the uterus, mammary gland, bone and cardiovascular system, whereas ER β is mainly expressed in non classical tissues such as the prostate, ovary and urinary tract. Within the brain ER expression appears species-specific [78]. In rodents, the presence of ER- α in brain regions such as the hypothalamus, amygdale and hippocampus, indicate a main role for ER- α in estrogen modulation of neuroendocrine functions, autonomic events and memory processing, whereas a higher level of expression of ER- β appears to be localized in the the basal forebrain and cerebral cortex [78].

Although some groups failed to detect ERs in the striatum or SN of mice at either the mRNA or protein levels, recent evidence of a relative abundance of ER- β in the SN and ventral tegmental area suggests that this receptor may be important in E₂ modulation of midbrain DA systems [79-81]. In particular, studies carried out in ER- β KO mice indicate the importance of this receptor for neuronal survival, since specific degeneration of neuronal cell bodies particularly evident in SN was reported in aged ER- β KO mice [82]. Furthermore using an ER- β selective antibody ER- β immunoreactivity was recently and primarily localized to cell nuclei within selected brain regions including the ventral tegmental area, and the SN [82].

On the othe other hand, it should be mentioned, that differences in reported studies may also depend on variations of ER subtypes according to the physiological condition studied, including gender and ageing, but also as a

function of the type (acute or chronic) of brain insult. Of special interest, as recalled in the previous sections, the increased expression of aromatase in activated astrocytes and the consequent increase in local E_2 synthesis in selected brain injured areas might have therefore a further impact in specific ERs expression of either neuron or glial populations.

The mechanism(s) responsible for E_2 neuroprotection have been studied in a wide variety of neuronal systems including the DA neuronal system, both *in vivo* and *in vitro*, and have been shown to involve a multitude of direct genomic and non genomic-mediated effects, including anti-apoptotic, growth factor-mediated, and antioxidant effects [83-88].

Generally, E_2 action takes place through signaling pathways involving the canonical activation of nuclear ER. This mechanism is implicated in the regulation of gene expression and requires direct interactions with palindromic sequences in the promoter region of target genes [89]. Estrogen bound-ER translocates into the nucleus and in conjunction with co-activators and co-repressors modulates gene expression. Additionally, non classical signalling (also termed non-genomic, rapid estrogen effect) involving extranuclear ERs (cytoplasmatic, plasma membrane-attached, and mitochondrial-located) plays an important role in mediating physiological estrogen effects [64]. Often, the activation of such alternative, non-nuclear ER-dependent pathways entails the stimulation of other intracellular signalling cascades such as the MPA-kinases, PI3-kinase, CaCaM-kinase, and protein kinase . Interestingly, ER-ligand complexes are reported to regulate the activity of NF-KB, an important transcriptional regulator of immune function (as discussed in the next sections). The exact molecular mechanisms of nonclassical estrogen signaling are not fully clarified, it seems apparent that classical ERs located at different intracellular sites are coupled directly or through specific adaptor proteins to these signalling cascades [89]. While some evidence reveals the requirement of ERs in estrogen mediating neuroprotection, the differential role of each subtype is not completely elucidated.

Using the MPTP mouse model which produces striatal DA denervation comparable to PD, the neuroprotective effects of 17- β estradiol and the mechanisms implicated in these effects have been studied by a number of laboratories [60-64]. Evidence from a number of experiments carried out *in vivo* indicate a genomic mechanism of action to explain the beneficial effects of estradiol on dopaminergic neurons. Indeed, the protection obtained with E_2 was only obtained with the β -isomer, while 17- α treatment did not afford neuroprotection [62]. Using chronic treatments with ER α and ER β agonists in neuroprotection against MPTP D'Astous et al. have pointed to ER α as critically implicated in protection against MPTP-induced DA neurotoxicity [62]. In female MPTP-treated mice, endogenous levels of E_2 or exogenous E_2

administration of ovariectomized females have protective effects on nigrostriatal DA neurons [25 and further sections]. Rat mesencephalic neurons cultured in an E₂-containing medium are resistant to apoptosis and neuronal injury, and this effect is blocked by the ER-antagonist ICI 182,780 [87,88]. Conversely, by using a coculture model of embryonic mesencephalic neurons and mesencephalic or striatal astrocytes, the crucial participation of astrocytes in E₂ neuroprotective effect against serum deprivation-induced cell death was clearly documented, suggesting indirect glial-mediated effects of the hormone [25].

Mounting evidence clearly indicates that besides the multitude of E₂-mediated effects in exerting neuroprotection, estrogen's anti-inflammatory mechanism(s) may play an important role in the MPTP mouse model of PD [25]. The central role of gender and estrogen in dictating major estrogen-dependent differences in both innate and adaptive arms of immunity will then be summarized. Indeed, involvement of E₂ modulation of glial neuroinflammatory reaction in the MPTP model of PD has recently emerged [22,25].

Gender, E₂ estrogen and sex dimorphism in innate and adaptive immune responses: Implications for neuronal vulnerability

The incidence and severity of human diseases is known to vary between sexes. For example autoimmune diseases are generally more common in females than in males and are most marked in women of childbearing age [90]. It seems apparent that susceptibility to autoimmunity is expressed at the time of puberty. Hormonal changes occurring at puberty induce fundamental biological differences that persist throughout life, thus contributing to the variable onset and progression of disease in males and females [3,91]. Sex-related differences in disease susceptibility have also been observed in several mouse models of infectious and autoimmune diseases and may be related to differences in the expression patterns of immune response genes. Indeed, immune responses are sexually dimorphic, both in type and magnitude and gender and the sex steroid hormonal milieu play a central role in the regulation of innate, cell-mediated and humoral immune responses outside the brain, thereby chiefly contributing to the development and/or severity of some autoimmune diseases such as lupus, multiple sclerosis (MS), Rheumatoid arthritis (RA), and Graves's disease [91]. Two general systems of immunity to infectious agents have been selected during evolution: innate (natural) immunity and acquired (adaptive or specific) immunity. The innate immune system uses proteins encoded in the germline (on macrophages, mast cells, dendritic cells and natural killer cells) to recognize conserved products of infectious non-self (i.e. microbial pathogens). In contrast to this relatively

inflexible system is the almost infinitively adaptable immune system of lymphocytes [90]. These two systems are known to closely interact with each others: for example cellular and soluble components of innate immunity help the adaptive immune response to select and respond to appropriate antigens. Of special importance, recent evidence clearly indicates a sexual dimorphism in innate and adaptive immune response genes as a function of puberty, implicating the changing sex steroid hormone milieu as a key factor in remodelling immune functions with the result that sexual dimorphism in immunity is permanently established in adult life [90]. For example, in both experimental animals and in humans, stimulus activated immunity is greater in females than in males, and antigen-presenting cells are more effective in females [91].

The effect of E_2 on immune cells and estrogen's influence on immune responses have been investigated both *in vitro* and *in vivo*. *In vivo* experimental studies indicate that E_2 influences many different cell types either directly or indirectly, including B cells, T cells, macrophages, and NK cells, stromal cells, and endothelial cells [91]. The *in vivo* effects of E_2 in immune parameters include altered cytokine production, cell differentiation, and expression of adhesion molecules [92,93]. Additionally, there is evidence that E_2 increases Th2 type cytokines and accordingly decreases cell-based immunity in both animals and human models [92-94].

In terms of local brain inflammation, this pattern of immune regulation would suggest a decrease in the expression of proinflammatory cytokines and a decrease in cell-mediated immunity. Many published reports have shown that physiological levels of E_2 significantly decrease microglia superoxide production, nitrite release or phagocytosis in response to stimulation by LPS, IFN- γ , MPTP intoxication, or the HIV protein [25,95-98]. The multifaceted regulatory effects of estrogens can be appreciated when studying the effects of estrogens upon microglial antigen presentation and T cell activation, documenting the hormonal ability to significantly decrease components of adaptive immunity, thereby importantly influencing microglia-T cell dialogue [99].

It should be underlined that the effects of E_2 can be direct effects on immune cells and/or modulate immune functions via neuroendocrine-immune interactions, also modulation of the HPA axis [see previous sections]. In addition, the effects of E_2 are, both dose- and cell-type specific on immune cells and depending on the specific physiological/experimental condition, E_2 can act in both a pro-inflammatory and anti-inflammatory manner.

It seems important to recall that in autoimmunity, Th1/Th2 balance and the activation of effector T cell subsets are often critical for the progression or the remission of disease, and E_2 might play a decisive role in changing the plasticity of these cells to drive the immune response towards the protection against disease. Thus sex steroids shift T helper cells towards a Th2 phenotype,

and cytokines produced by Th2 cells generally suppress EAE [94]. Indeed, ERs are present in T cells, macrophages and dendritic cells at all stages of differentiation, and direct E₂ modulatory effects have been demonstrated. It is worth noting that E₂ can potentiate the production of anti-inflammatory Th2 cytokines, IL-10 and TGF-beta, in antigen-specific T cells, resulting in full protection against EAE. Coupled to the recently reported effect of E₂ in driving the expansion of the "regulatory" T cell compartment [100], these data clearly suggest the possibility that E₂ may dampen inflammation by directly influencing Th1/Th2 balance. It is still not clear, however, whether a similar mechanism is operating within the CNS. In fact, signalling from activated cells in the periphery to the brain might be also influenced by E₂. Neuroimmune signalling involves besides others, neuroendocrine-immune interactions, (especially the stress axis), NO, circulating cytokines, which interact with brain endothelium and macrophage populations in the brain, and in turn, these signals are passed on to particular neuronal populations leading to mild and chronic brain cell inflammation. For some neurodegenerative diseases, a correlation between ongoing central and peripheral inflammation and increased susceptibility to neuronal death has been clearly demonstrated [23].

Significantly, the onset of menopause has been associated with a spontaneous increase in cytokine production, specifically TNF- α and the interleukins IL-1 and IL-6. Moreover, cytokines levels are reportedly lower in post-menopausal women [101] on hormone replacement therapy and in estrogen-treated ovariectomized mice compared to untreated controls. Within the brain, age-related increases in glial activation as well as age-related increase in cytokines and their receptors documented by histology and gene expression analysis indicate a widespread inflammatory response [102,103]. Aging is also associated with functional alterations of the blood brain barrier (BBB) [104], which may also result in increased permeability with serum leakage and leucocyte infiltration into the neuronal parenchyma. It seems also important to underline that besides age-induced pro-inflammatory status, pre-existing inflammatory conditions, such as giant cell arthritis and systemic lupus erythematosus, increase predisposition to neurodegenerative diseases, as do a range of acute and chronic infections, principally respiratory [23].

Collectively, these data indicate the ability of E₂ to powerfully modulate both innate and adaptive immune mechanisms outside and within the brain. Although E₂ modulation of immune homeostasis is a complex phenomenon with E₂ playing a dual pro- and anti-inflammatory role, the process of ageing and estrogen deficiency may alter the Th1/Th2 balance, with an increase in pro-inflammatory status. In addition E₂ deficiency, as reviewed in the next section may impact on astrocyte-microglial dialogue, with important implications for neuronal vulnerability to a number of acute and chronic brain insults/conditions, including PD.

Neuroinflammation, Parkinson's disease, glia and hormones

Accumulating evidence clearly suggests a pivotal role for glia and neuron-glia crosstalk both in the adult and aging brain and E_2 has been implicated in the modulation of both astrocytes and microglial cell function. In the central nervous system (CNS), astroglial cells are recognized as playing active roles in both health and disease states and in influencing the development of the brain's response to a variety of insults [23]. Astrocytes which represent the major glial population and microglial cells, like other tissue macrophages, are key components of the neuroendocrine-immune axis and, as such, are responsive to endocrine, neural and immune factors. In particular, astrocytes, and macrophages/microglial cells express hormone receptors (i.e. glucocorticoid receptors, GRs and estradiol receptors, (ERs), and are both a source of and target for cytokine, growth and neurotrophic factor activities in the brain [22,24,25].

Although developmental and constitutive functions of the majority of these immunoregulatory molecules in the physiology of the normal, immune privileged CNS are still not generally accepted, when CNS homeostasis is disturbed as a result of a trauma, stroke, ischemia, infection or degenerative processes, certain cytokines increase, as a result of blood-brain-barrier disruption, or from local synthesis by invading immune cells. Then, most, if not all neuropathologies are associated with glial cell activation and reactive gliosis recognized as a universal hallmark of both acute or chronic damage to the CNS [23]. Thus, activated astrocytes and microglia serve as endogenous sources of various cytokines involved in the orchestration of cellular responses aimed at rapid re-establishment of tissue integrity and subsequent repair [23]. Release of products of activated glia are thought to be important for initiating and guiding the infiltration of immune cells and for coordinating their activities in the brain tissue. While glial responses may be considered beneficial mechanisms, when local production of cytokines exceeds the appropriate range, cytokine-mediated neurotoxicity may lead to severe neuronal damage [105-109]. Activated microglial cells are also important participants in local cell-mediated immunity, as activated microglia are not only phagocytic, but are also potent sources of reactive oxygen and nitrogen species and excitotoxins.

Hence, a wide variety of inflammatory mediators once thought to be restricted to peripheral immune responses are now considered to be central to the pathogenesis of major neurodegenerative diseases. A full innate immune system (e.g. complement system, scavenger receptors, Toll-like receptors) has been described in the CNS and is thought to be an extremely efficient system to fight against invading pathogens and toxic cell debris. There is abundant evidence that a number of proinflammatory mediators, such as cytokines, and

inflammatory-associated factors such as cyclooxygenase-2 (COX-2) and inducible-nitric oxide synthase (iNOS), are elevated in the CNS or cerebrospinal fluid of Huntington's disease (HD), Alzheimer's (AD), PD, multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) patients [23]. With regard to PD, the first evidence for the involvement of inflammation in PD dates back to 1988, when McGeer and coworkers described the up-regulation of major histocompatibility complex (MHC) molecules in PD brains. Later, Mogi and coworkers reported increased levels of beta2-microglobulin, the light chain of MHC, in the striatum of PD patients [17,18]. Accumulation of ROS, NO, COX-2 products and pro-inflammatory cytokines (including TNF- α , IL-1 β and IFN- γ) in the SN of PD patients further supported the hypothesis that a state of chronic inflammation characterizes the PD brain [11-17]. Confirming a role for cytokines, inactivation or genetic ablation of TNF- α receptors attenuated cell death in the MPTP model of PD [13-17,23]. NOS also seems important, as shown by demonstration of a cytokine/CD23-dependent activation pathway of iNOS, and by the association between PD and NOS polymorphisms [11,27]. COXs also appears to play a role, as shown by increased COX-2 expression in the SN of PD patients [23]. Epidemiological evidence has shown that chronic NSAIDs reduce the risk of PD by about 45% [20]. On this basis, several studies have been carried out in the MPTP model of PD with non-selective and selective COX-blockers. However, discrepancies between these studies (likely depending on use of different administration protocols and *in vivo* modalities of MPTP treatment) make it difficult to draw any conclusion. Several other different treatments, such as immunological approaches, use of peroxisome proliferator activator receptor gamma agonists (PPAR γ), or compounds containing the basic morphine, or flavonoid/phenolic structure proved to be beneficial [23].

Collectively, these findings point to neuroinflammation as a therapeutic target in PD neurodegeneration, with astrocytes and microglia being key players in this scenario. On the other hand, in view of the dual *destructive/neuroprotective* role of activated astrocytes and microglia, the manipulation of glial *beneficial* and *pro-regenerative* capacities appears central in providing novel therapeutic opportunities for PD [23-25]. Consequently, the active search for endogenous and exogenous astroglial "regulators" in PD appears of prime importance.

Astrocytes, microglia, hormones and the MPTP model of PD: A pivotal role for estrogen in modulating neuron-glia crosstalk

Besides others, one endogenous glial regulatory feedback mechanism may be represented by hormonal background, in particular estrogens are known

regulators of astrocytes, macrophage/microglial and endothelial cell function [22,24,25]. In addition, infiltrating T cells and peripheral macrophages are critically modulated by the sex steroid hormone background [4]. There are also crucial interactions between the stress hormones and E_2 in modulating the response of inflammation [see previous sections].

There is abundant evidence that cultured astrocytes and microglia possess estrogen receptors [69]. A number of laboratories have investigated the effects of estrogens in LPS-stimulated astrocyte, macrophage or microglial cells. Of major interest, estrogens are potent inhibitors of inducible nitric oxide (iNOS)-derived NO in activated astrocytes and macrophage/microglial cells [22,25,95-99, 110]. Consistently, the primary sex steroid hormone, E_2 , may play an active role under in vivo conditions in which increased CNS levels of cytokines would have several adverse consequences. In fact, during inflammation, it seems highly possible that a sophisticated interplay between hormones of the stress and reproductive axes [22,24,25,31,37] participates to the temporal and spatial correct expression of iNOS/NO in different organs and tissues, leading to the elimination of inflammatory agents with minimal tissue damage.

It is worth noting that within the brain, E_2 via either ER-alpha and/or ER-beta, has been shown to exert anti-inflammatory activity on activated macrophages and activated microglial cells in vitro, as revealed by the prevention of lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines including TNF-alpha, iNOS-induced NO, COX-2, prostaglandin-E2 (PGE2), and metalloproteinase-9 (MMP-9) [95-99]. Most importantly, E_2 can interact with the transcription factor NF-kB, a key regulator of inflammatory responses [98]. Together with the recently described E_2 effects of in microglia-T cell dialogue [99], this information clearly emphasizes E_2 modulation of brain immune homeostasis as a determining factor for dictating neurodegeneration on neuroprotection.

MPTP is a known neurotoxicant inducer of parkinsonism in human patients that has subsequently been modelled extensively in mice as well as non human primates [8,9]. MPTP crosses the blood brain barrier and is subsequently converted to the neurotoxic metabolite MPP^+ , a substrate of the dopamine transporter (DAT). Astrocytes represent a primary locus for MPTP metabolism, and experimental data have clearly documented a key role for glial cells in MPTP metabolic activation and MPP^+ delivery into the extracellular compartment [8]. Once inside DA neurons, MPP^+ inhibits complex I of the mitochondrial electron transport chain, resulting in ATP depletion and subsequent neuronal death (Fig. 2). This energy crisis initiates a chain of events culminating in the inability to release a sufficient amount of dopamine, locomotor deficits, and ultimately apoptosis of tyrosine hydroxylase-positive (TH^+) neurons in the SN [10]. Although the sequence

of events is not clearly understood, MPP⁺ toxicity stimulates the generation of oxygen-free radicals, activates astrocytes and microglia to produce a number of pro-inflammatory cytotoxic mediators, such as cytokines and NO (Fig. 2).

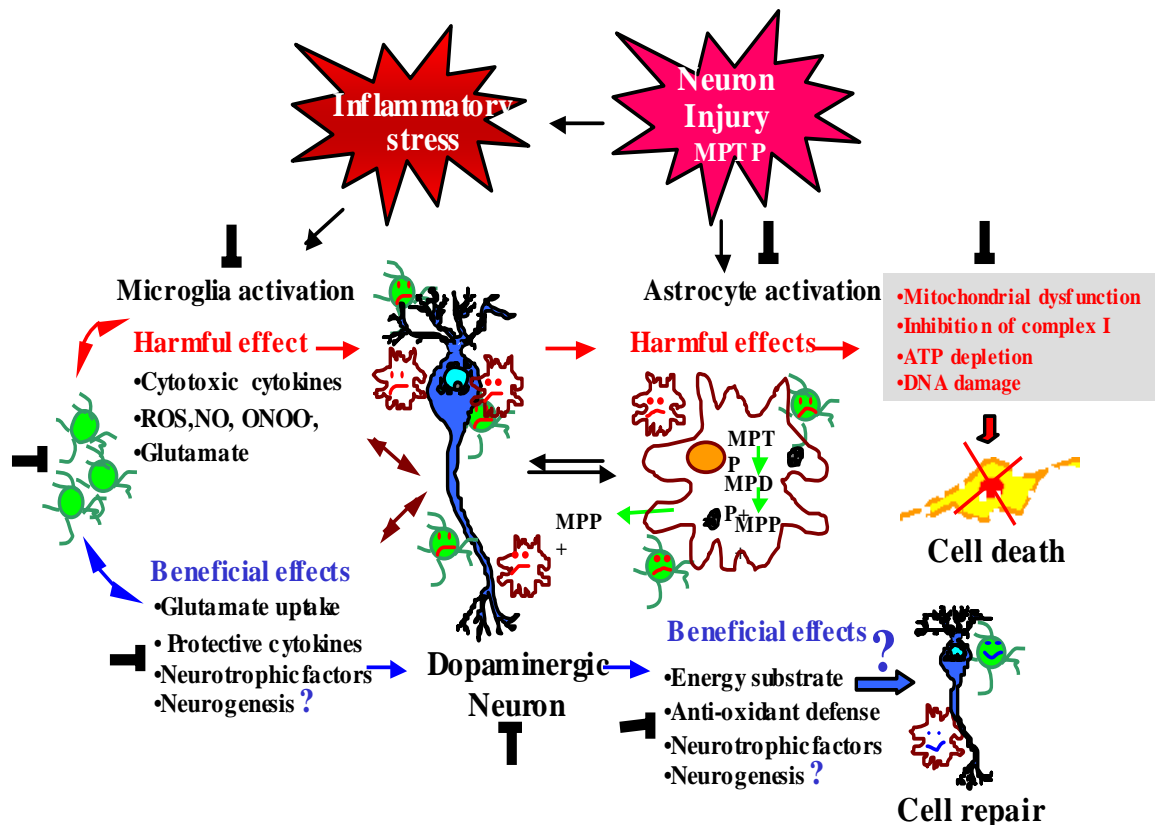


Figure 2. A schematic representation of detrimental and beneficial inflammatory pathways in PD. DA neurons in the substantia nigra pars compacta (SNc) represent a preferential target for inflammatory stressors, due to enzymatic and non enzymatic autooxidation of DA generating H₂O₂, to the high toxicity of DA metabolites, and the interactions between iron (which is highly concentrated in SNc) and H₂O₂ in the Fenton reaction, leading to highly toxic radicals. Injury of these neurons, as a result of specific chemical insults (e.g., MPTP), and/or a combination of genetic/environmental factors (viruses, endotoxin, pesticides, susceptibility factors) leads to marked glial cell activation. MPTP is metabolized by astroglia to 1 methyl-4-phenylpyridinium (MPP⁺) which is concentrated in DA neurons. Here it inhibits cellular respiration, generates oxygen-free radicals and NO and initiates a cascade of cytotoxic events culminating in reduced DA release, locomotor deficits, and ultimately cell death by apoptosis. Astrocytes, which express glutathione at high levels, can protect neurons by scavenging radicals and glutamate, by harboring receptors for endogenous anti-inflammatory molecules (such as GCs and E₂), by providing energy support, trophic factors, «protective» cytokines and possibly by stimulating repair processes. On the other hand, under conditions of chronic inflammatory stress, activated astrocytes and microglia may become dysfunctional and over-express a variety of cytotoxic mediators eventually resulting in DA neuron death [see text, for details]. The hypothetical role of endogenous glucocorticoids (GCs) and estrogens (E₂) in “switching” the “harmful” into a “beneficial, protective” glial phenotype, is illustrated.

Consequently, oxidative stress may be linked to other processes such as mitochondrial impairment, inflammation, excitotoxicity and the toxic effects of NO [8-10,14]. NO can promote oxidative damage to macromolecules including cellular proteins implicated in PD pathogenesis such as tyrosine hydroxylase (TH) and alpha-synuclein. Then, MPTP intoxication triggers both oxidative and nitrosative cytotoxic cascades (the “Mr Hyde” face of glia) largely contributing to nigral DA neuron demise. Astrocytes are known to play a central role in the antioxidant defense of the brain and to exert a variety of neuroprotective functions. Astrocytes scavenge reactive oxygen species (ROS) released by neurons and remove glutamate from the extracellular space, thereby reducing the exposure of NMDA receptors to this excitatory amino acid; they produce factors that induce antioxidant enzymes, and express crucial neurotrophic molecules, regulating growth, differentiation and survival of neurons, as part of the bidirectional, neuronal-glia interactions [111-116]. In particular, fetal mesencephalic neurons in culture are extremely vulnerable to serum deprivation [111] or damaging compounds such as peroxynitrite, as compared to cultured glial cells, whereas interaction between astrocytes and neurons has been variously demonstrated to exert striking neurotrophic, differentiation and neuroprotective effects [25].

Glial cells play a pivotal role in all MPTP-toxic mechanisms: a. they contribute to H_2O_2 production (via the MAO-B-dependent dopamine and MPTP metabolism); b. they represent a primary locus for H_2O_2 and reactive nitrites scavenging; c. they provide energy support for neurons in conditions of metabolic stress; d. they increase GSH export via up-regulation of Mrp1 efflux pump [117]; e. they exert “anti-inflammatory” effects via GR and ERs activation and down-regulation of iNOS/NO and NF- κ B antagonism [25]; and f. they protect against NMDA receptor activation by the active uptake of glutamate. However, an imbalance of oxidative and nitrosative cascades triggered by MPTP may damage astrocytes/impair glial functionality, in that astroglial cells may become less efficient and lose their defensive and neuroprotective capacities [25] (Fig. 2).

Together, these findings clearly implicate both astrocytes and microglia in the neurotoxic response to MPTP, the potential role of E_2 in “switching” the “harmful” into a “beneficial, protective” glial phenotype helping nigrostriatal DA neuron rescue will be then discussed in the next sections.

The estrogenic status modulates the response of the glial cell compartment to the neurotoxin MPTP: Implications for nigrostriatal neuron vulnerability to PD

As in normal menstruating women, in normal cycling female rodents, fluctuations in plasma E_2 levels occur. The cyclic changes in plasma E_2 have been reported to modulate structural parameters in various brain regions. In

particular, region-specific changes of astrocyte and microglia morphology, cell number and or immunoreactivity, have been reported to occur, *in vivo*, as a function of gender and the estrous cycle, aging, gonadal hormone deprivation and E₂ replacement [118-123]. In addition, sex steroids, in particular E₂, have been demonstrated to play a major role in modulating changes of glial reactivity, and/or proliferation occurring after brain injury [69]. In female rodents, various parameters of nigrostriatal system functionality, including dopamine concentration, dopamine uptake sites and DA1 receptor density, have been shown to vary according to the phases of the estrous cycle (see previous sections and Introduction).

Based on this literature, the endogenous hormonal status at the time of injury may differentially impact on MPTP-induced DA toxicity and/or on the ability of nigral neurons to recover. Hence, changes in astrocyte and microglia cell function may underlie the neurochemical and morphological alterations of nigrostriatal DA neurons as a result of estrogen cyclic variation, as well as after estrogen loss and estrogen replacement. We recently addressed whether changes in estrogenic status might alter the astroglial response to MPTP underlying estrogen neuroprotection against nigrostriatal DA neurotoxicity [25]. To this end, temporal changes in different indices of glia reactivity (immunocytochemistry and generation of iNOS-derived NO) and DA neuron functionality (striatal dopamine and its metabolites) were assessed after injection of the neurotoxin, MPTP [25], at proestrous or estrus corresponding to high and low plasma E₂ levels, respectively, after bilateral ovariectomy (OVX, performed 2-3 weeks before MPTP treatment), both in the absence or the presence of a concomitant treatment with 17-beta (E₂, 1 ug/day) or 17-alpha (1 ug/day) estradiol. In accordance with our previous studies [31], time-course analysis of the effect of MPTP on the astroglial cell compartment indicated that early (1 day) after MPTP treatment, immunolabeling for GFAP started to increase in striatum and midbrain of all MPTP-treated groups, but such an increase was different according to the estrogenic status [25]. Lowest astrocyte reaction was observed in intact female mice treated with MPTP at proestrous, corresponding to the phase of highest E₂ serum levels, or in OVX mice supplemented with E₂, whereas maximal astrocyte hypertrophy was observed in E₂-deprived (OVX) mice, when plasma E₂ levels were almost undetectable [25]. Induction of a microglial reaction in the denervated striatum and SN of female mice was also earlier and sharper in OVX as compared to intact MPTP-treated mice. In addition, one day after MPTP injection in cycling females at proestrus, iNOS-IR was weak. By contrast, in OVX mice, a strong iNOS-IR signal was localized in both the striatum and SN in both GFAP-IR and GFAP-negative round-to-oval shaped cells, identified as activated macrophages/microglia [25]. Accordingly, up-regulation of the nitrosative stress foot print, nitrotyrosine, and NADPH-diaphorase were revealed in the

SN of OVX, as opposed to intact mice at this early time-point. In addition, 3 days after MPTP, a significant increase in GFAP-IR astrocytes and TH-IR neuron death by Terminal deoxynucleotidyl transferase-mediated 2'-deoxy-uridine-5'-triphosphate nick end labelling (TUNEL), was observed in estrogen deprived, but not intact mice [25]. By contrast, treatment with E₂, but not 17-alpha estradiol, sharply inhibited iNOS-IR and decreased TUNEL and NADPH-diaphorase reactions in both the striatum and SN [25].

Collectively, the findings indicate that cyclic fluctuations of plasma E₂ levels importantly impact on the glial response to MPTP in female mice, with a significant reduction of astrocyte, microglial and iNOS nigrostriatal activation when the neurotoxin is administered during maximal estrogenic activation, whereas E₂ deficiency as a result of OVX sharply increases all indices of glia activation which is accompanied by increased nitrosative stress and programmed cell death of both nigral astrocytes and DA neurons, confirming a key role for endogenous E₂ in dampening the harmful glial reaction while protecting astrocyte-mediated beneficial effects [25].

Estrogen deficiency up-regulates microglial iNOS/NO response to MPTP: Implications for the demise of nigrostriatal DA neurons

A number of studies including our own demonstrated a determining role for macrophage/microglia iNOS/NO response (measured by its decomposition product nitrite) to MPTP-induced DA neurotoxicity [24,25,31]. The hypothesis was put forward that estrogen deficiency-induced increase in brain nitrinergic status/sensitivity may impact on DA neurotoxicity induced by MPTP. Evaluation of the effect of the estrogenic status on NO generation from brain macrophage/microglial cells isolated at different time-intervals after MPTP intoxication, and correlation of those levels with decreases of striatal dopamine implicated such a mechanism [25]. Indeed, although we found that in all mice groups, microglial nitrite generation increased as a result of MPTP injection in a time-dependent fashion, such increases were however significantly different according to estrogenic status and preceded the decrease of DA and its metabolites. Consequently, as early as after 6 hrs from MPTP, OVX mice exhibited a 4- to 5-fold increase in nitrites as compared to intact estrous, proestrous mice or OVX mice treated with E₂, but not 17-alpha estradiol [25]. It should be noted that decreases of striatal dopamine and its metabolites (DOPAC+HVA) were first observed 24 h after MPTP, and that such decreases were far greater in OVX (-68 and -61%, respectively) as compared to estrus (-42 and -38%), proestrus (-28 and -24%), or OVX mice treated with E₂ (-19 and -17%), indicating exacerbation of DA neurotoxicity as a result of ovarian hormone withdrawal, and a greater protection afforded by the hormonal

background of proestrus [25]. Accordingly, within the intact mice group, between 6 and 24 h, microglial nitrite levels increased, but such levels were significantly ($p < 0.01$) higher in mice treated with MPTP at estrus, as compared to mice at proestrus, indicating that endogenous estrogenic status sharply modulates microglia functional iNOS/NO response to MPTP. In addition, higher microglial NO levels of OVX mice preceded the greater depletion (-86%) of striatal dopamine and synaptosomal [^3H] dopamine uptake (almost 25% of controls) measured 11-28 days after MPTP, as opposed to intact mice showing a significant degree of functional recovery ([^3H]dopamine uptake: almost 67% of control). Thus, proestrus E_2 levels, or exogenous E_2 administration are associated with down-regulation of MPTP-induced iNOS-derived nitrites, reversal of astrocyte death, reduced DA toxicity and stimulation of DA functional recovery, whereas OVX exacerbates astrocyte hypertrophy, microglia iNOS/NO generation and subsequent astrocyte and DA toxicity. These findings support a prominent protective role for circulating sex steroids against MPTP-induced DA neurotoxicity, and further show an important effect of E_2 proestrus levels at the time of MPTP injection [25].

In summary, from the overall findings, it seems tempting to speculate that a causal relationship may exist between the exacerbation of glia reactivity of E_2 -deprived females and increased vulnerability of nigral DA neurons to MPTP, possibly implicating E_2 -induced switch of pro-inflammatory astroglial "Mr Hyde" into a "Dr Jekyll" phenotype, as a determining factor in dopaminergic neuron protection. This complementary action of estrogen on astrocyte and microglia herein reviewed in the MPTP model of PD, may provide a potential pharmacological target and a new insight into the therapeutic potential of these hormones in Parkinson's disease.

Conclusion

In conclusion, although E_2 is best known for its effects on the maturation and differentiation of the primary and secondary sex organs, increasing evidence clearly suggests that its influence extends beyond this system, and its activity in the CNS may initiate, or influence our susceptibility to neurodegenerative decline. Indeed, the overall accumulated evidence clearly underlines hormonal ability to act as a neuroprotectant at several levels. While E_2 intrinsically produced within the brain may act as a survival and differentiation factor at selected perinatal time-windows, increased aromatase expression in brain injured astrocytes coupled with the consequent increase of the hormone in specific lesioned-regions may significantly contribute to decreased neuronal vulnerability as well as to neuronal recovery and or repair. Long-term deprivation of circulating E_2 and a decrease of brain aromatase activity as a result of menopause exposes the aging or diseased brain to several insults, including increased risk/incidence of PD. In addition, E_2 deprivation is likely to initiate or enhance a

pro-inflammatory status both at a systemic and central level. Moreover, estrogen deficiency may be linked to the dysfunction of astrocyte and microglial compartments, with reduced ability of astrocytes to perform their crucial antioxidant and defensive functions, resulting in altered glial-neuron crosstalk. Decreased function of astroglial cells, e.g. as a result of increased oxidative/nitrosative stress, may lead to reduced expression of growth and neurotrophic factors, reduced scavenging properties and reduced anti-oxidant enzymes production, with consequent damage to neurons and inhibition of repair processes. With regard to the implication for PD, the findings herein reviewed in the MPTP model of PD suggest that endogenous E₂ participate in the modulation of astrocyte and microglia reactivity and iNOS inducibility during neuroinflammation induced by MPTP. In particular, ovulatory E₂ levels appear to contribute to restrain astroglial cell response to nitrosative stress, and consequently protect dopaminergic neurons against the described cytotoxic cascades, whereas estrogen deprivation sharply up-regulates various indices of astrocyte and microglia activation resulting in exacerbation of DA neurotoxicity. It seems interesting to notice that intact female mice appear more resistant when MPTP is administered on proestrous, as compared to females treated at estrus. On the other hand, the hormonal background of estrus is able to significantly protect striatal indices of DA functionality against MPTP neurotoxicity as compared to OVX mice. Furthermore, intact female mice exhibit a significant degree of recovery from DA neurotoxicity, both at morphological and functional levels, thereby supporting a protective role for circulating ovarian hormones.

Given the crucial role of glia-neuron interactions in neuronal growth, survival differentiation and synapse formation, as well as in modulation of neurogenesis [124], alterations in glia-neuron crosstalk, as observed in E₂-deprivation conditions, suggest a pivotal role for endogenous E₂ in restraining harmful innate inflammatory reactions, thereby contributing to enhance astroglial neuroprotective functions and possibly helping the repair process (Fig. 3).

Consistently, it should be underlined, that while activation of innate immunity in the CNS accompanied by neurodegeneration may significantly impair neurogenesis, neuroinflammatory blockade and astrocyte “beneficial” activation can restore this crucial process [124-128]. In summary, the herein described estrogenic activation of glial anti-inflammatory and “protective” functions may provide a further mechanism reducing the detrimental effects of neuroinflammation, while promoting cytokine activation of astroglial “pro-regenerative” functions. This mechanism might represent a compensatory/adaptive response to stimulate the repair process (Fig. 3). Although the accumulating evidence from basic science studies using animal models suggests that E₂ plays a critical neuroprotective role against multiple types of neurodegenerative diseases and injuries, recent clinical studies have reported either inconclusive or untoward side

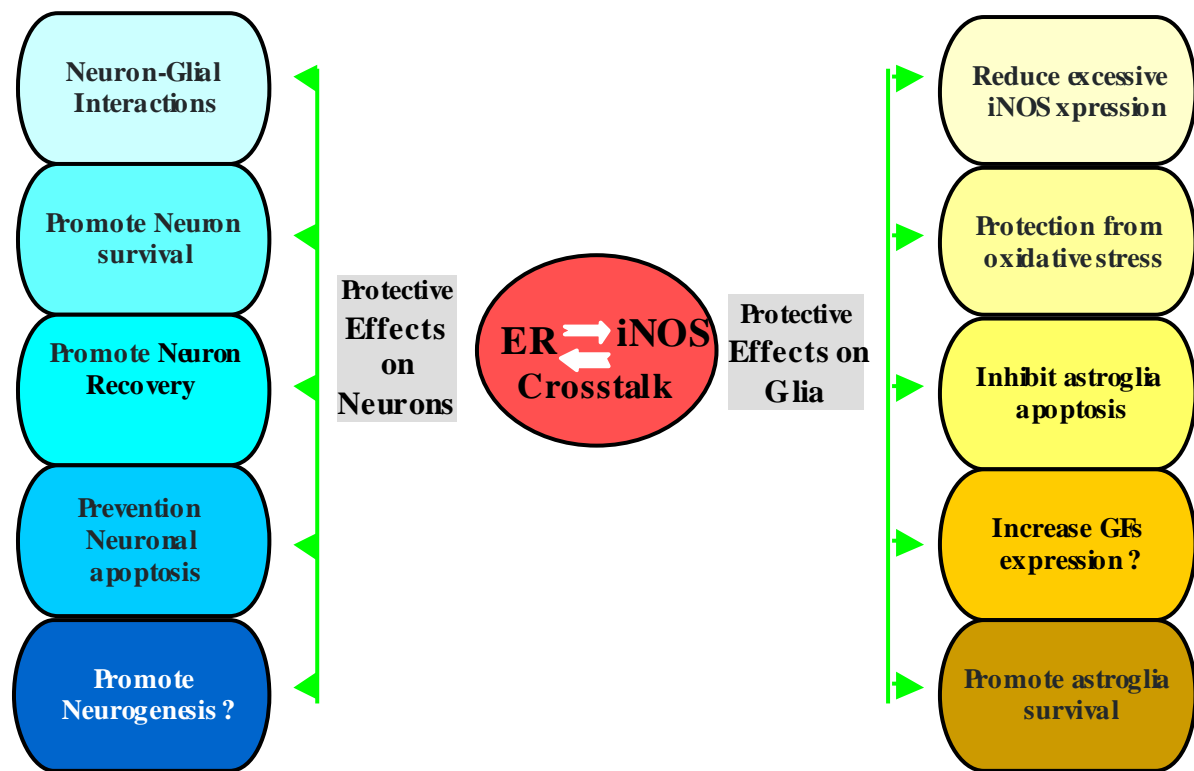


Figure 3. Schematic representation of potential estrogen receptor/inducible-nitric oxide (iNO) crosstalk in glial neuroprotective functions. Stimulation of innate immunity has bidirectional effects on activated astroglial cells resulting in pro- and anti-inflammatory cascades with positive and negative influences on neuronal survival/protection/rescue. Activation of glial ER-iNO crosstalk may provide a further endogenous mechanism reducing neuroinflammatory detrimental effects, while promoting astroglial “pro-regenerative” functions [25].

effects of hormone therapy on the brain [46]. Indeed, results from the Women's Health Initiative Memory Study (WHIMS) clinical trial have induced a reconsideration of the efficacy of estrogen hormone therapy as a strategy to prevent age-related cognitive decline and dementia, and a number of important issues, still remain unresolved, such as the timing and the duration of estrogen therapy as well as the combined presence of progestins. Development of selective receptor modulators for the brain (SERMs), also called NeuroSERMs, certainly represent one major pharmacological challenge, to target E_2 -specific subtype receptors, in order to prevent brain-related climateric symptoms and neurodegenerative diseases [129-132]. The complementary action of estrogen on astrocyte and microglia herein reviewed in the MPTP model of PD, may provide a potential pharmacological target and a new insight into the therapeutic potential of these hormones in Parkinson's disease, and a further means to design “bifunctional” molecules aimed at preserving on the one hand astrocyte functionality while, on the other, restraining the aberrant stimulation of innate immunity.

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