

## Review

# Tipping Points and Endogenous Determinants of Nigrostriatal Degeneration by MPTP

Stefan Schildknecht,<sup>1,\*</sup> Donato A. Di Monte,<sup>2</sup> Regina Pape,<sup>1</sup> Kim Tieu,<sup>3</sup> and Marcel Leist<sup>1</sup>

The neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a Parkinson's disease (PD)-like syndrome by inducing degeneration of nigrostriatal dopaminergic neurons. Studies of the MPTP model have revealed the pathomechanisms underlying dopaminergic neurodegeneration and facilitated the development of drug treatments for PD. In this review, we provide an update on MPTP bioactivation and biodistribution, reconcile the distinct views on energetic failure versus reactive oxygen species (ROS) formation as main drivers of MPTP-induced neurodegeneration, and describe recently identified intrinsic features of the nigrostriatal system that make it particularly vulnerable to MPTP. We discuss these new perspectives on the endogenous tipping points of tissue homeostasis and the drivers responsible for vicious cycles in relation to their relevance for the development of novel intervention strategies for PD.

### MPTP: An Experimental Parkinsonian Toxicant

Almost four decades ago, the neurotoxicant MPTP was identified as an illicit drug contaminant that can cause symptoms and signs in humans similar to those observed in idiopathic PD [1]. Studies during the 1980s elucidated the basic mechanisms of toxicity of MPTP, which has since become the most-studied experimental neurotoxicant. It is now textbook knowledge that the protoxicant MPTP is metabolized by astrocytic **monoamine oxidase-B** (MAO-B; see [Glossary](#)) to generate the active metabolite 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) ([Figure 1](#)). MPP<sup>+</sup> is taken up into neurons by **dopamine (DA) transporters** (DATs) [2–5]. Cytosolic MPP<sup>+</sup> is accumulated in catecholaminergic storage vesicles by **vesicular monoamine transporter-2** (VMAT-2) and in mitochondria through membrane potential-dependent uptake [6]. MPP<sup>+</sup> accumulates in the mitochondrial matrix and inhibits complex I of the respiratory chain, leading to an impairment in mitochondrial ATP generation and an increase in superoxide (•O<sub>2</sub>-) formation [6–8]. Furthermore, MPP<sup>+</sup> uptake into DA neurons triggers vesicular DA release, which results in DA autoxidation and free radical formation ([Figure 2](#)). In the presence of iron and H<sub>2</sub>O<sub>2</sub>, extracellular DA can be oxidized to form 6-hydroxy-DA. This toxicant can undergo cyclization to yield aminochrome, which is capable of directly inhibiting complex I [9]. Thus, oxidative stress and energy failure are involved intricately in a vicious cycle. ATP depletion triggered by MPP<sup>+</sup> emerged as insufficient to trigger cell death [10]. A growing body of evidence indicates a more significant role of ROS generation as consequence of complex I inhibition than initially anticipated [11,12]. For instance, mice overexpressing copper/zinc superoxide

### Trends

PD-associated motor deficits are characterized by nigrostriatal dopaminergic-neuron loss.

The parkinsonian protoxicant MPTP is activated in the central nervous system (CNS) by astrocytic monoamine oxidase-B to form the active toxicant 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>).

Membrane-impermeable MPP<sup>+</sup> is taken up by neurotransmitter transporters into catecholaminergic neurons.

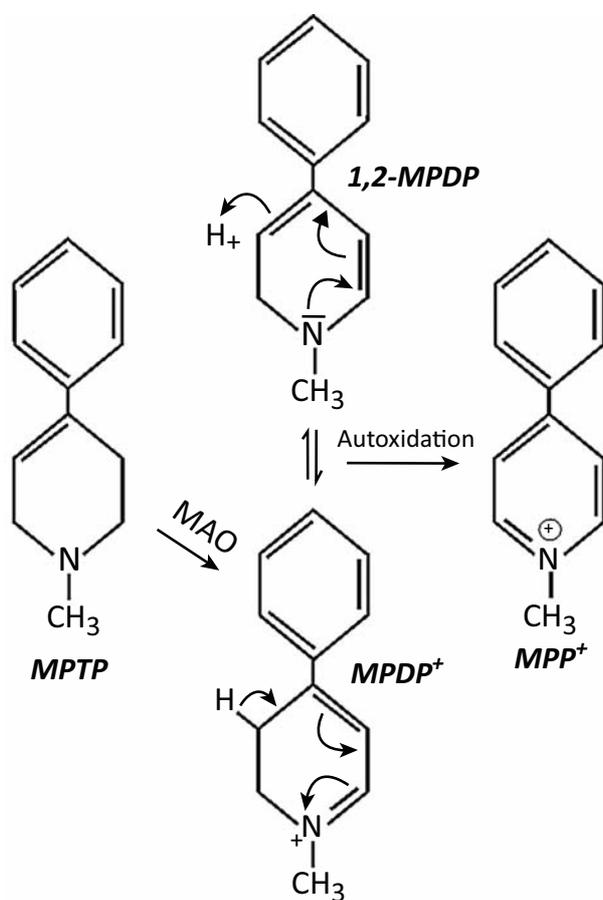
Inside cells, MPP<sup>+</sup> inhibits mitochondrial complex I, reducing mitochondrial ATP output and favoring ROS formation.

In MPTP-exposed brains, different catecholaminergic populations show large sensitivity differences, with a distinct nigrostriatal dopaminergic-neuron degeneration, reflecting the degeneration pattern in PD.

Intrinsic factors, such as MPP<sup>+</sup> uptake and/or vesicular-sequestration kinetics, neuronal morphology, and intracellular Ca<sup>2+</sup> handling, distinguish sensitive from resistant neuronal subpopulations in the MPTP model and in PD.

<sup>1</sup>In vitro Toxicology and Biomedicine, Department of Biology, University of Konstanz, D-78457 Konstanz, Germany

<sup>2</sup>German Center for Neurodegenerative Diseases (DZNE), Sigmund-Freud-Strasse 27, D-53127 Bonn, Germany

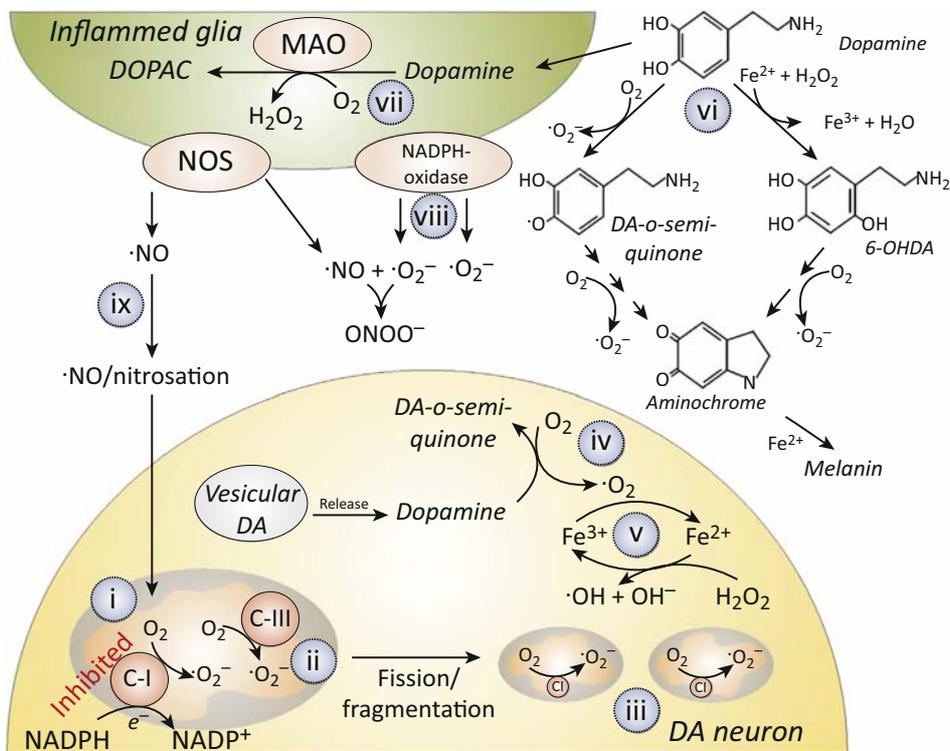


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Figure 1. Structures of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) and Its Metabolites. The intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP<sup>+</sup>) is generated through a two-electron oxidation, catalyzed by monoamine oxidase (MAO). Under physiological conditions, MPDP<sup>+</sup> partially exists in its conjugate base form, 1,2-MPDP. Given that it is lipophilic and uncharged, 1,2-MPDP can readily cross membranes without the need for a transporter. In a second, nonenzymatic step, the intermediate undergoes autoxidation to form the stable active toxicant 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>).

dismutase (Cu/Zn-SOD) displayed higher resistance against MPTP [13], while knockdown of endogenous Cu/Zn-SOD elevated the sensitivity of nigrostriatal DA neurons towards MPTP [14] (Figure 3).

Recent groundbreaking studies on MPTP models have provided data that cannot be satisfactorily explained by standard textbook knowledge regarding MPTP toxicokinetics and mode of action. Take, for example, modulation of neuroinflammation-induced damage: blockade of IL-1 $\beta$ , TNF- $\alpha$ , or IFN- $\gamma$  signaling prevented MPTP neurotoxicity, despite ongoing MPP<sup>+</sup> inhibition of mitochondrial complex I [15–17]. Another example is related to mitochondrial fission and/or fusion. Genetic silencing or pharmacological suppression of dynamin-like protein-1 (DLP-1), a mitochondrial fission-associated molecule, prevented mitochondrial dysfunction and neurodegeneration, despite MPP<sup>+</sup>-dependent inhibition of mitochondrial complex I [18–21]. Moreover, adenosine A<sub>2A</sub> receptor antagonists were reported to protect against MPTP-triggered neurodegeneration, but the underlying mechanisms remain inadequately characterized [22]. Another set of questions arises from the cell type specificity of MPTP and/or MPP<sup>+</sup>-induced damage: motor deficits in PD and in MPTP models correlate closely with nigrostriatal DA-



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**Figure 2. Free Radical Sources.** 1-Methyl-4-phenylpyridinium (MPP<sup>+</sup>) primarily acts as an inhibitor of mitochondrial complex I (C-I). At this site, MPP<sup>+</sup> exposure stimulates  $\bullet\text{O}_2^-$  generation (i). Elevated free radical levels can lead to oxidative modifications of C-I and of mitochondrial complex III (C-III) (ii). This increases and perpetuates  $\bullet\text{O}_2^-$  formation by the respiratory chain. Consequently, mitochondrial fragmentation is accelerated, leading to increased  $\bullet\text{O}_2^-$  formation by fragmented mitochondria (iii). MPP<sup>+</sup> also triggers vesicular dopamine (DA) release into both the cytosol and the synaptic cleft. Cytosolic DA undergoes autooxidation, which increases  $\bullet\text{O}_2^-$  formation (iv). The  $\bullet\text{O}_2^-$  radical can be dismutated to form  $\text{H}_2\text{O}_2$ , which, in the presence of iron, can initiate the Fenton reaction and drive the Haber-Weiss cycle to form highly reactive hydroxyl radicals ( $\bullet\text{OH}$ ) (v). Extracellular DA can also undergo autooxidation and thereby favor  $\bullet\text{O}_2^-$  formation. In the presence of iron and  $\text{H}_2\text{O}_2$ , extracellular DA is oxidized to form the toxicant 6-hydroxy-DA (6-OHDA), whose subsequent cyclization generates aminochrome and  $\bullet\text{O}_2^-$  (vi). Aminochrome can directly inhibit C-I and, thus, leads to a self-perpetuating cycle. Extracellular DA, accumulated by astrocytes, can be detoxified by glial monoamine oxidase (MAO), a reaction that involves  $\text{H}_2\text{O}_2$  generation (vii). Glial cells, activated by inflammatory stimuli or degenerating neurons, can serve as a potent source of  $\bullet\text{O}_2^-$ , formed by, for example, NADPH oxidases (viii). Glial cells and neurons are also sources of nitric oxide ( $\bullet\text{NO}$ ), generated by either constitutively expressed NO synthase (NOS)-1 or inducible NOS-2 (ix). A fitting example of reactive oxygen species (ROS) function is the finding that mice were protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity after overexpression of the antioxidative enzyme Cu/Zn-superoxide dismutase (Cu/Zn-SOD); conversely, endogenous Cu/Zn-SOD knockdown elevated DA neuron MPTP sensitivity. However, the relative contribution of different ROS sources warrants further consideration when interpreting the results obtained using diverse MPTP/MPP<sup>+</sup> models and extrapolating the results to PD. Mitochondrial ROS generation is a more complex cell biological process than previously assumed: MPTP and MPP<sup>+</sup> lead to mitochondrial fission through oxidant effects on various proteins, such as dynamin-like protein-1 (DLP-1), and mitochondrial fragmentation increases ROS output. Notably, the tipping points of all processes linked to oxidative stress and disturbed cellular proteostasis are dependent on counter-regulations (antioxidants, chaperones, etc.). These differ strongly between experimental systems (even from mouse strain to mouse strain), which helps to explain why the literature is often apparently contradictory, with certain findings being challenging to reproduce, and why predictions from models to the human disease are associated with large uncertainty.

neuron degeneration and the concomitant decline in striatal DA levels [23]. This has obscured the finding that MPTP also markedly affects DA neurons of the mesocortical system or hypothalamic tuberomammillary nucleus [24]. Furthermore, DAT-mediated toxicant uptake does not explain why MPTP spares, for example, DA neurons of the ventral tegmental area

#### Glossary

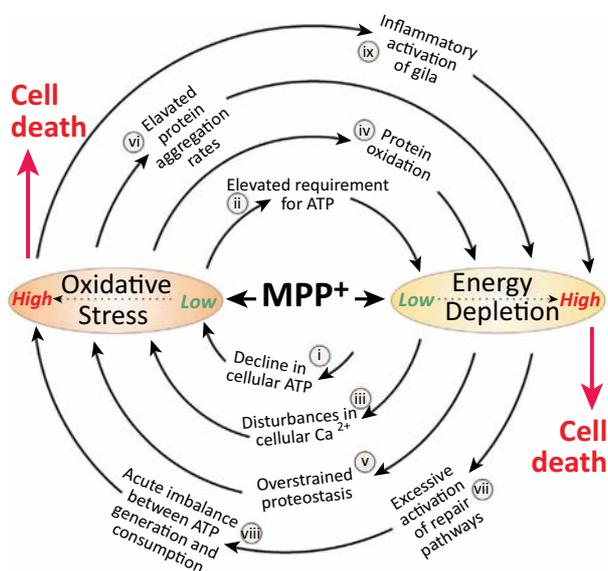
**Cytochrome P450 oxidase (CYP2D6):** involved in the metabolism and elimination of xenobiotics.

**Dopamine transporter (DAT):** expressed by dopamine neurons; enables re-uptake of synaptic dopamine to terminate its signaling function. DAT also allows uptake of MPP<sup>+</sup>, thus contributing to its preferential toxicity in these neurons.

**Monoamine oxidase (MAO):** catalyzes the oxidative deamination and inactivation of monoaminergic neurotransmitters. MAO also catalyzes the activation of MPTP to form the toxicant MPP<sup>+</sup>.

**Organic cation transporter (OCT):** allows passive transport of organic cations (incl. MPP<sup>+</sup>) across membranes.

**Vesicular monoamine transporter 2 (VMAT-2):** transport of neurotransmitters and MPP<sup>+</sup> from the cytosol into synaptic vesicles.



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**Figure 3. 1-Methyl-4-Phenylpyridinium (MPP<sup>+</sup>) As a Trigger of Vicious Cycles.** Binding of MPP<sup>+</sup> to complex I inhibits mitochondrial ATP synthesis and triggers  $\bullet\text{O}_2^-$  formation (i). These events initiate a series of self-amplifying and self-perpetuating processes that lead to the demise of the cell through a vicious cycle. To compensate for elevated  $\bullet\text{O}_2^-$  formation, the cell must maintain antioxidant systems, which further burdens the energy budget (ii). As a result of  $\text{Ca}_v1.3$ -mediated pacemaking, nigrostriatal dopamine (DA) neurons face a constant influx of extracellular  $\text{Ca}^{2+}$ . Inappropriate control of cellular  $\text{Ca}^{2+}$  pools, as a consequence of an already stressed ATP budget (iii), can lead to  $\text{Ca}^{2+}$  accumulation in mitochondria and thereby to an increase in mitochondrial  $\bullet\text{O}_2^-$  generation. Such elevated rates of ROS formation lead to increased rates of oxidative modifications of proteins, lipids, and DNA (iv), which further reduce key cellular functions, such as mitochondrial ATP synthesis. Moreover, cellular functions such as proteostasis are reduced as a consequence of inappropriate ATP generation (v). This leads to an accumulation of oxidatively modified and misfolded proteins (vi), which further compromises mitochondrial function. Activation of cellular repair pathways leads to an additional demand for ATP (vii), further enhancing the already existing imbalance between ATP consumption and generation (viii). All of the aforementioned events ultimately result in the initiation of cell damage or death, and this leads to an inflammatory activation of surrounding glial cells. Glial-derived reactive oxygen and nitrogen species further increase oxidative stress in the remaining neurons (ix).

(VTA). MPP<sup>+</sup> uptake by other catecholamine transporters explains the pronounced degeneration of noradrenergic neurons of the locus coeruleus in the MPTP model [25], but is inconsistent with the resistance of serotonergic, MAO-B-containing neurons [26].

These examples of outstanding questions and inconsistencies illustrate how the simplified textbook view is insufficient for explaining the observations made using MPTP models (see Outstanding Questions). Conversely, new insights into MPTP metabolism and biodisposition, together with expanding knowledge regarding the intrinsic features of neuronal subpopulations primarily affected in the MPTP model and PD, have led to a more precise understanding of the underlying mechanisms. Here, we review these novel findings to facilitate the interpretation of data obtained using MPTP and/or MPP<sup>+</sup> models, and address the critical question of how observations made in these test systems can broaden our understanding of pathogenetic processes in PD.

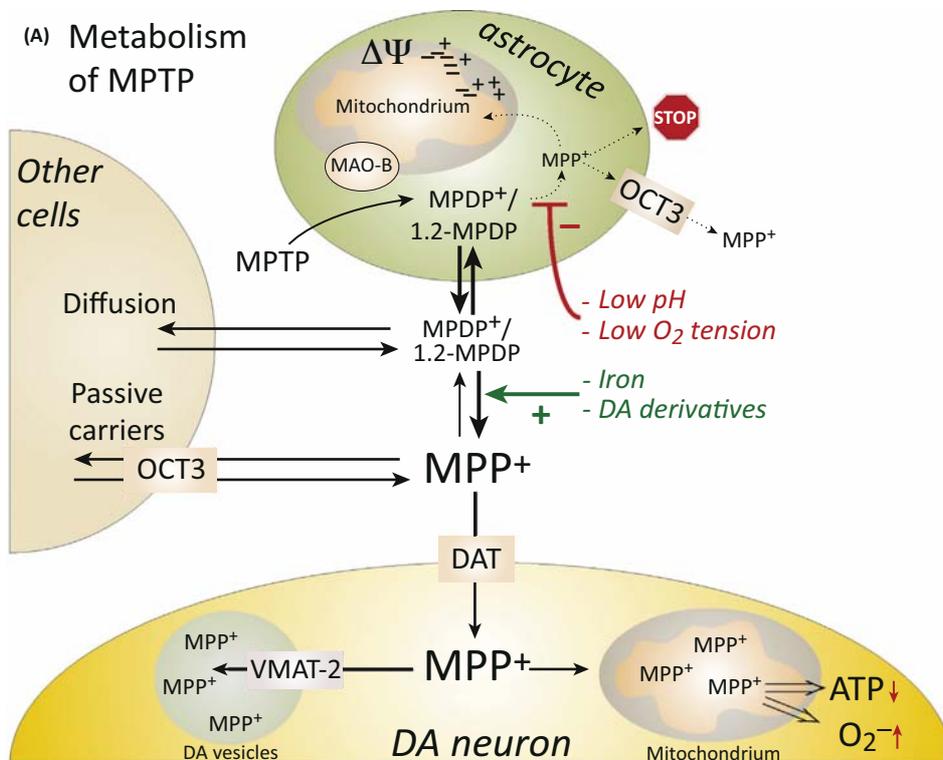
### Why Is MPP<sup>+</sup> Not Accumulating within Astrocytes?

In the central nervous system (CNS), MPTP is converted to MPP<sup>+</sup> almost entirely by MAO-B, a mitochondrial outer-membrane enzyme expressed predominantly in astrocytes and, to a minor extent, in serotonergic neurons [27,28]. Being charged, MPP<sup>+</sup> cannot diffuse across cell

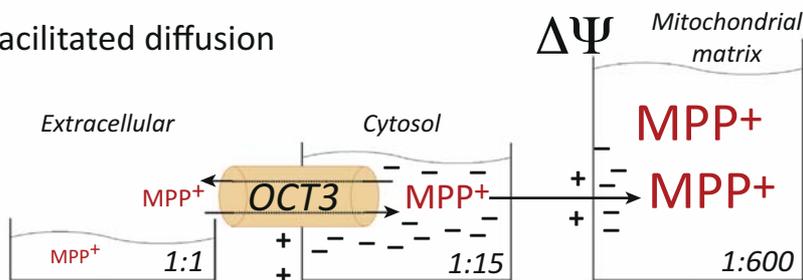
membranes, and would be expected to accumulate and cause toxicity within MPP<sup>+</sup>-producing cells. This contrasts with the lack of astroglial damage typically observed upon MPTP exposure [29,30], although astrocytes are not MPP<sup>+</sup> resistant. In astrocytes, genetically modified to express DAT, MPP<sup>+</sup> accumulates intracellularly and elicits a toxic response comparable to that in DA neurons [31,32]. These findings suggest that MPP<sup>+</sup> toxicity in any cell type is determined mainly by the toxicant concentration reached inside mitochondria. Cells have been widely demonstrated to exhibit distinct susceptibilities largely due to variations in MPP<sup>+</sup> uptake across the plasma membrane, availability of export mechanisms, and intracellular deposition in organelles. Astrocytes might be spared from MPTP-induced cell death because of an efficient export of MPTP metabolites [32] (Figure 4).

One key carrier that enables MPP<sup>+</sup> equilibration across cellular membranes is the **organic cation transporter-3** (OCT-3) [33]. OCT-3 is preferentially expressed in glial cells located near the DA neurons, and OCT-3-deficient mice were highly resistant to MPTP toxicity [33]. This suggests a critical role of OCT-3 in MPTP-dependent neurodegeneration, although the mechanistic basis is complex because OCT-3 allows not only the export of cationic compounds, such as MPP<sup>+</sup> or monovalent paraquat, but also their uptake [33,34]. Depending on the brain region and phase of MPTP metabolism, OCT-3-expressing cells might act as a transient 'sink' for high levels of extracellular MPP<sup>+</sup>, or OCT-3 might allow MPP<sup>+</sup> efflux from cells in which MPP<sup>+</sup> is highly accumulated. This complex MPP<sup>+</sup> distribution is also affected by the relative affinities of the transporters, as exemplified by the  $K_m$  of DAT being lower than that of OCT-3 (Figure 4). Near DAT-expressing neurons, this lower  $K_m$  of DAT for MPP<sup>+</sup> favors MPP<sup>+</sup> uptake and retention within dopaminergic neurons [33].

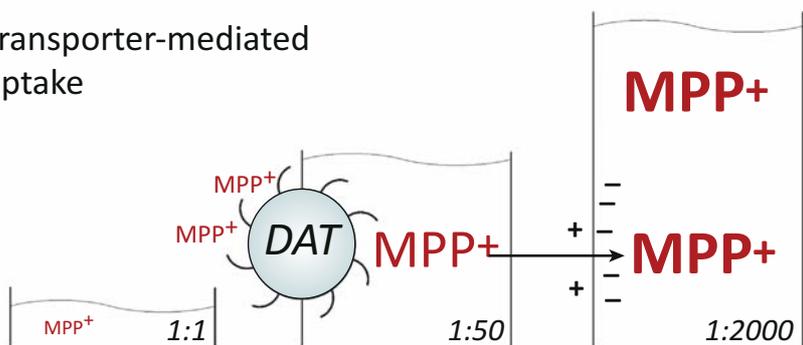
MPTP metabolites are also exported through a transporter-independent mechanism [32]. Understanding how MPP<sup>+</sup> is removed from astrocytes lacking OCT or related transporters requires comprehension of the metabolic steps involved in MPTP bioactivation. This two-step process starts with MAO-B-catalyzed two-electron  $\alpha$ -carbon oxidation of MPTP, which generates the unstable intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP<sup>+</sup>) [35,36]. In the second step, which is nonenzymatic, MPDP<sup>+</sup> undergoes autoxidation to form the stable MPP<sup>+</sup> [37] (Figures 1 and 4). Although a role of MPDP<sup>+</sup> in extracellular MPP<sup>+</sup> formation and accumulation was suggested in early MPTP studies [3,37], MPDP<sup>+</sup> was only recently measured in brain tissue and identified as a transporter-independent export metabolite in its uncharged, membrane-permeable free-base form: 1,2-MPDP [32]. Specific conditions in brain tissues have been shown to stabilize the intermediate intracellularly and concurrently promote its conversion to MPP<sup>+</sup> extracellularly. The intracellular milieu is characterized by lower oxygen tension due to mitochondrial consumption and moderately more acidic conditions compared with the extracellular environment [38]. Whereas these conditions prevent MPP<sup>+</sup> formation when MPDP<sup>+</sup>/1,2-MPDP is inside cells, conversion into MPP<sup>+</sup> is promoted by the more alkaline and oxygen-rich environment and the iron-containing complexes in the extracellular space [32] (Figure 4). Identification of MPDP<sup>+</sup>/1,2-MPDP as a membrane-permeable MPTP metabolite and elucidation of the extracellular conditions that favor its autoxidation to MPP<sup>+</sup> provide a convincing mechanism for explaining the resistance of MPTP-converting astrocytes (Figure 4). The rapid clearance of extracellular MPP<sup>+</sup> out of the brain guarantees a constant steep concentration gradient between intracellular and extracellular compartments and, thus, a sustained efflux of MPDP<sup>+</sup>/1,2-MPDP from astrocytes. This molecular explanation for preferential MPP<sup>+</sup> formation in the extracellular space is also required for understanding the mechanism underlying the long-recognized DAT-dependent uptake of extracellular MPP<sup>+</sup> into nigrostriatal DA neurons [32]. The notion that a large fraction of MPP<sup>+</sup> formed in the brain prevails in the extracellular space is supported by microdialysis and mass spectrometry studies in MPTP-exposed mice and rats [39,40]: MPP<sup>+</sup> levels rose rapidly throughout the brain, but MPP<sup>+</sup> was also cleared rapidly (1–2 h)



(B) Facilitated diffusion



(C) Transporter-mediated uptake



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Figure 4. Metabolism of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) and Distribution of 1-Methyl-4-Phenylpyridinium ( $MPP^+$ ). (A) In the brain, most MPTP is converted by glial monoamine oxidase-B (MAO-B) into the intermediate  $MPDP^+/1,2-MPDP$ , which freely diffuses across biological membranes. Once outside astrocytes, the intermediate might either diffuse into other cells or undergo nonenzymatic autoxidation to form the stable toxicant  $MPP^+$ . Several factors favor a preferential formation of  $MPP^+$  in the extracellular space: inside cells, the relatively

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in most areas, except regions rich in neurons that actively accumulate MPP<sup>+</sup> via their catecholamine transporters.

### Which Enzymes in Neurons or Astrocytes Contribute to MPTP Oxidation?

Chiba *et al.* [35] discovered the MAO-dependent activation of MPTP to form MPP<sup>+</sup>. Knockout studies and pharmacological intervention studies using MAO-A-/MAO-B-selective inhibitors indicated that MAO-B has a dominant role in MPTP activation in the CNS [41]. An alternative activation pathway, MPTP conversion by **cytochrome P-450 2D6** (CYP2D6), was also suggested [42,43]. Indeed, genetic association studies revealed a link between a polymorphism at the locus *CYP2D6* and PD susceptibility [44]. However, biochemical activity studies indicated that high CYP2D6 activities were correlated with a diminished risk of PD [45,46]. A potential molecular explanation for these observations is the detoxification of endogenously formed  $\beta$ -carbolines and isoquinolines and inactivation of pesticides by CYP2D6 [42,47].

The aforementioned observations in PD raise this question: does CYP2D6 have a protective or toxicity-enhancing role in the MPTP model? The major metabolites produced by CYP2D6, 1-methyl-4-(4-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (OH-MPTP; *p*-hydroxylation) and 4-phenyl-1,2,3,6-tetrahydropyridine (PTP; *N*-demethylation) [47], are not neurotoxic, and only approximately 10% of MPTP is transformed into MPDP<sup>+</sup> [48]. This agrees with the finding that the cytochrome P450 inhibitor diethyldithiocarbamate increases MPTP toxicity in mice [49]. Furthermore, CYP2D6 upregulation protected against MPTP also *in vitro* [50]. Analysis of the respective contributions of MAO-B and CYP2D6 to MPTP oxidation revealed that the oxidation rate by MAO is approximately threefold higher [48]. The fact that CYP2D6 has a minor role only in MPP<sup>+</sup> formation is supported by results obtained using MAO-B-knockout mice, which were almost completely protected against MPTP [41]. Thus, even if CYP2D6 contributes to MPDP<sup>+</sup> formation, the extent of this reaction would likely be insufficient to damage and kill DA neurons. In conclusion, CYP2D6 expression in the nigrostriatal system can be regarded as a protective factor in idiopathic PD and in the MPTP model.

### How Does Sequestration inside Neurons Determine MPP<sup>+</sup> Toxicity?

Efficient inhibition of isolated complex I of the mitochondrial respiratory chain was found to require 10–20 mM MPP<sup>+</sup> [6], concentrations that are 500–2000-fold higher than those measured in brain homogenates after MPTP treatment [51,52]. This raises the question of how MPP<sup>+</sup> is accumulated intracellularly at concentrations sufficient for complex I inhibition (Figure 4).

lower pH and low oxygen tension contribute to a stabilization of the intermediate, whereas in the extracellular space, the availability of either catalytically active iron-containing complexes or dopamine (DA) autoxidation products can strongly accelerate the autoxidation of MPDP<sup>+</sup> into MPP<sup>+</sup>. Extracellular availability of MPP<sup>+</sup> is a prerequisite for its cell specific, transporter-dependent accumulation in catecholaminergic neurons. In DA neurons, cytosolic MPP<sup>+</sup> is either actively accumulated in neurotransmitter vesicles by vesicular monoamine transporter-2 (VMAT-2), or is enriched in the mitochondrial matrix, driven by the mitochondrial transmembrane potential. In mitochondria, MPP<sup>+</sup> inhibits complex I of the respiratory chain, which leads to the limitation of mitochondrial ATP production and to an increase in  $\bullet\text{O}_2$  formation at complex I. MPP<sup>+</sup> distribution in the brain is also affected by passive transporters, such as organic cation transporter-3 (OCT-3). Such bidirectional transporters allow a membrane potential-dependent uptake of MPP<sup>+</sup>. Under conditions of low extracellular MPP<sup>+</sup>, these transporters also facilitate the export of intracellular MPP<sup>+</sup>. (B) Transport of extracellular MPP<sup>+</sup> into the mitochondrial matrix requires passage across the (1) cell membrane and the (2) inner mitochondrial membrane. Passive transporters allow an intracellular accumulation of the cation MPP<sup>+</sup>, according to the Nernst equation. At a given membrane potential of  $-70$  mV, an approximately 15-fold concentration of MPP<sup>+</sup> can theoretically be achieved. (C) Active transporters, such as the DA transporter (DAT), enable a unidirectional build-up of MPP<sup>+</sup> in the cytosol against a concentration gradient by a factor of approximately 1:50. In a second step, cytosolic MPP<sup>+</sup> is accumulated in the mitochondrial matrix, mostly driven by the transmembrane potential of the inner mitochondrial membrane. This step permits an additional concentration by a factor of approximately 1:40. When extracellular MPP<sup>+</sup> is present in the low micromolar range, OCT expression is inadequate to enable quantitative complex I inhibition, whereas at the same concentrations, DAT expression allows millimolar toxicant concentrations to be reached in mitochondria, which is sufficient for inhibiting the respiratory chain.

When passive transporters are present, the plasma membrane potential of  $-70$  mV would lead to an approximately 15-fold concentration of the monovalent cation  $MPP^+$  intracellularly, according to the Nernst equation [32]. Moreover, isolated respiring mitochondria accumulate  $MPP^+$  by a factor of approximately 40 [6]. Given that tissue  $MPP^+$  concentrations of  $>10$   $\mu$ M can be reached in standard MPTP models *in vivo* [51,52], this two-step electrochemical accumulation would allow  $MPP^+$  to reach millimolar concentrations in the mitochondrial matrix (Figure 4). However, such concentrations would be maintained only transiently: MPTP intoxication is characterized by rapid  $MPP^+$  clearance from the brain (half-life in the 1-h range). Moreover, cytosolic  $MPP^+$  efflux through a bidirectional carrier would reduce its mitochondrial concentration, which would lead to the sparing of cells from  $MPP^+$  toxicity.

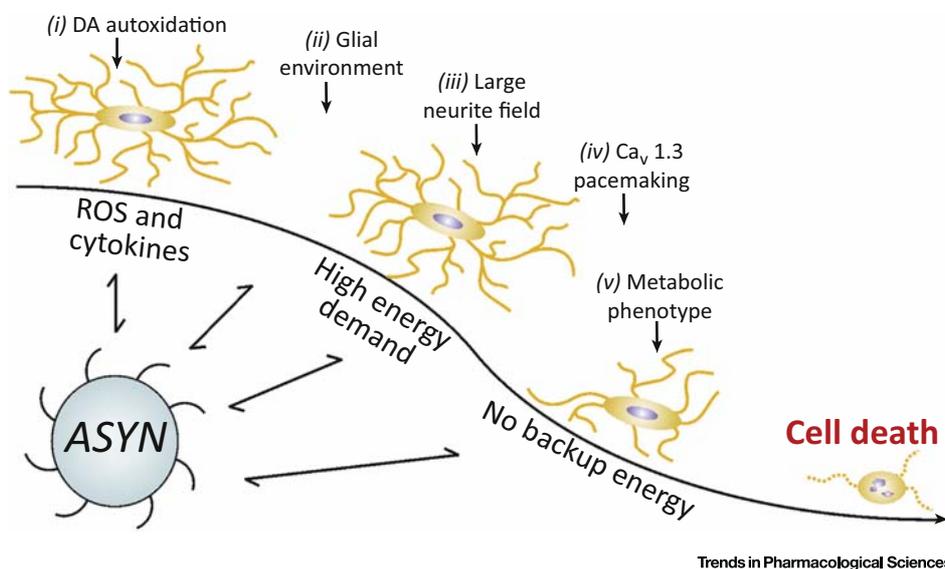
The aforementioned scenario is contrasted by what occurs in catecholamine neurons, which express two active transporters: a plasma membrane catecholamine transporter, such as DAT; and the vesicular transporter VMAT-2. Unlike the OCT-transporter-mediated bidirectional passive flux, catecholaminergic transporters drive active, unidirectional  $MPP^+$  accumulation (Figure 4). Once  $MPP^+$  is in the cytosol, its further sequestration, either by mitochondrial accumulation or by VMAT-2-mediated vesicular accumulation, largely determines  $MPP^+$  toxicity. Whereas DAT overexpression enhanced neuronal MPTP sensitivity [53], pharmacological inhibition of DAT protected against MPTP toxicity [5]. VMAT-2 mediates vesicular  $MPP^+$  sequestration, which prevents  $MPP^+$  toxicity and, accordingly, VMAT-2 overexpression resulted in diminished toxicity [54], whereas its genetic ablation or pharmacological inhibition elevated the MPTP sensitivity of DA neurons [55]. Analysis of DAT and VMAT-2 expression in distinct neuronal populations revealed that the DAT:VMAT-2 ratio was higher in MPTP-sensitive striatal terminals of nigral DA neurons than in comparatively less sensitive VTA neurons [56]. Disturbances in vesicular storage affect not only DA neurons, but also noradrenergic neurons of the locus coeruleus. In a low-VMAT-2-expression mouse model, locus coeruleus noradrenergic neurons exhibited progressive degeneration even before the onset of nigrostriatal DA neurodegeneration [57]. Conversely, polymorphisms identified in the human VMAT-2 promoter region indicated that elevated VMAT-2 expression correlated with reduced PD risk [58,59]. Notably, mice exposed during development to dieldrin, an environmental toxicant, displayed an increased DAT:VMAT-2 ratio in the adult nigrostriatal system and, consequently, were more vulnerable to MPTP toxicity than were unexposed control mice [60]. In conclusion, not only the activity of cell surface catecholamine transporters, but also intracellular vesicular sequestration determines the widely recognized variations in MPTP sensitivity of distinct catecholaminergic populations of the brain. These factors must be carefully considered when interpreting the region-selective differences in neuronal degeneration observed in MPTP models.

### Features of Nigrostriatal DA Neurons That Affect the Tipping Point of $MPP^+$ Toxicity

As described above, mechanisms of  $MPP^+$  production, accumulation, and sequestration are not the only determinants of the MPTP susceptibility of neuronal subpopulations. This concept is exemplified by the evidence that not all catecholaminergic neurons (i.e., not all neurons that can take up  $MPP^+$ ) are equally targeted by MPTP neurotoxicity [23,24,26]. Specific neuronal features have recently been identified as modulators of the selective effects of MPTP, and these susceptibility factors could also have a critical role in PD pathogenesis.

#### Intracellular $Ca^{2+}$ and Its Influence on Energy Expenditure

Among the endogenous factors that contribute to selective neuronal degeneration in the MPTP model, dysregulation of intracellular  $Ca^{2+}$  homeostasis has a central role [61] (Figure 5), a conclusion supported by evidence from studies illustrating a neuroprotective influence of intracellular  $Ca^{2+}$  regulation [62]. Accordingly, the endogenous  $Ca^{2+}$ -binding protein calbindin appears to promote neuroprotection: MPTP-resistant VTA neurons express higher levels of



**Figure 5. Endogenous Susceptibility Factors of Nigrostriatal Dopamine (DA) Neurons.** Nigrostriatal DA neurons are continuously exposed to a series of stressors, such as elevated levels of free radicals, derived from DA autoxidation, (i) and from reactive oxygen and nitrogen species, which can be generated upon inflammatory activation of microglia in response to initial neuronal damage (ii). Nigrostriatal DA neurons differ from several other neuronal populations by virtue of their large total neurite length, a higher degree of neurite branching, and, consequently, a large number of synapses per neuron (iii). This leads to a particularly high energy demand, which is further increased because nigrostriatal DA neurons are autonomous pacemakers and use  $\text{Ca}_v1.3$  L-type  $\text{Ca}^{2+}$  channels for pacemaking (iv). Maintenance of intracellular  $\text{Ca}^{2+}$  homeostasis represents a continuous challenge to the cellular ATP budget. The individual metabolic phenotype (i.e., the high reliance on mitochondrial metabolism instead of glycolysis) further compromises DA neuron survival (v). None of these factors individually compromises the viability of healthy neurons, but their combination increases the susceptibility to any additional exogenous stressor. This is also the case with the highly abundant protein  $\alpha$ -synuclein (ASYN), which is expressed widely throughout the nervous system. Whereas the native protein most likely performs antioxidant and protective functions, it engages in multiple reciprocal interactions with cytosolic DA, elevated calcium, or surrounding glia, and the unique combination of ASYN,  $\text{Ca}_v1.3$ , and DA might represent a major susceptibility factor for nigrostriatal neurons.

calbindin than do neurons in the MPTP-sensitive substantia nigra pars compacta (SNpc), which agrees with the possibility that calbindin confers resistance against degeneration in the MPTP model [63]. Notably, the ranking of DA neuron susceptibility in the retrorubral area (A8), substantia nigra (A9), and VTA (A10) inversely correlates with calbindin expression in the respective regions in both mice and monkeys [64]. Moreover, the same relative ranking is found in PD and in the human brain, and correlates with the pattern of calbindin expression [63,65].

A major development in research on the relationship between  $\text{Ca}^{2+}$  homeostasis and selective neurodegeneration was the identification of an autonomous pacemaking activity of SNpc DA neurons and its reliance on the L-type  $\text{Ca}^{2+}$  channel  $\text{Ca}_v1.3$  [66,67]. PD is associated with cell loss in not only the SNpc, but also the locus coeruleus and hypothalamic tuberomammillary nucleus [68]. Intriguingly, these other two regions also exhibit autonomous pacemaking activity, again mediated by  $\text{Ca}_v1.3$  [69,70]. These observations reveal a potential correlation between the use of extracellular  $\text{Ca}^{2+}$  for pacemaking and elevated sensitivity both in PD and in MPTP models. For instance, olfactory bulb dopaminergic neurons are relatively spared from degeneration in PD and in the MPTP model; unlike SNpc cells, these neurons express T-type  $\text{Ca}^{2+}$  channels and do not rely on oscillatory  $\text{Ca}^{2+}$  waves for pacemaking activity [71]. Analysis of the pacemaking activity of VTA neurons, a population that displays higher resilience than does its nigrostriatal counterpart, indicated their reliance on  $\text{Na}^+$  instead of  $\text{Ca}^{2+}$  channels [72].

Involvement of extracellular  $\text{Ca}^{2+}$  for pacemaking is demanding metabolically, because export of the divalent ion  $\text{Ca}^{2+}$  requires a higher ATP investment per charge than does the use of monovalent ions [61,73]. A correlation between pacemaking-dependent energy expenditure and MPTP vulnerability was elegantly demonstrated in a cell model in which endogenous  $\text{Ca}_v1.3$  was either pharmacologically inhibited or genetically ablated; both conditions resulted in a 'rejuvenation' of neurons, which indicated that their pacemaking activity switched from a dependence on  $\text{Ca}_v1.3$  to a dependence on voltage-gated  $\text{Na}^+$  channels [74]. These rejuvenated neurons were considerably more resistant to MPTP/MPP<sup>+</sup> or rotenone toxicity than were their  $\text{Ca}_v1.3$ -expressing counterparts [74,75]. Collectively, these observations identified  $\text{Ca}_v1.3$  channels as potential targets for therapeutic intervention in PD. Notably, human data on how chronic treatment with  $\text{Ca}^{2+}$ -channel blockers might affect PD development are already available, because these compounds are frequently administered to treat patients with hypertension. Analysis of these initial clinical and epidemiological data indicated that PD risk was reduced in patients chronically treated with  $\text{Ca}^{2+}$ -channel blockers [76,77].

#### Morphology of SNpc DA Neurons As a Determinant of MPP<sup>+</sup> Toxicity

The pacemaker-associated oscillatory  $\text{Ca}^{2+}$  influx is not the only intrinsic feature underlying increased energy demand: the unique architecture of SNpc DA neurons, which had long been largely ignored, is currently suggested to represent another essential feature that contributes to selective neurodegeneration in PD and in the MPTP model [78] (Figure 5). Nigrostriatal DA neurons are characterized by unmyelinated, highly branched axons that can have a total length of up to 0.5 m (500 000  $\mu\text{m}$ ) per human neuron [79,80]. To maintain such long projections, neurons must expend a high amount of energy, which predisposes the neurons to an energy crisis in the presence of metabolic inhibitors. Moreover, the number of synapses formed by nigrostriatal DA neurons is at least two orders of magnitude higher than that formed by the less vulnerable DA neurons in the VTA [78,80,81]. The rat SNpc contains approximately 12 000 DA neurons [80], each of which forms approximately 100 000–250 000 synapses; in the human brain, this is another tenfold higher (1 million–2.4 million synapses per neuron) [78,82]. A computational model of nigrostriatal DA neurons indicated that the neurite-tree size in combination with the high synapse number represents a notable burden for the cellular energy budget: the energy cost increases exponentially with the total length and the degree of branching of the synaptic field [83]. Consequently, nigrostriatal DA neurons continuously face an energy demand that is barely met by the oxidative capacity of their mitochondria [84]. These anatomical features, in addition to the high energy requirement created by  $\text{Ca}_v1.3$ -mediated influx of extracellular  $\text{Ca}^{2+}$ , render SNpc DA neurons energetically 'on the edge' [78]. In such a scenario, even moderate stressors could lead to an imbalance of cellular energy provision and consumption (Figures 3 and 5). The concept of a low residual energy capacity could also provide a mechanistic basis for explaining the selective loss of nigrostriatal DA neurons in models of systemically applied rotenone, which, unlike MPP<sup>+</sup>, is not selectively accumulated in defined cell types.

#### Synergy of Alpha-Synuclein and Other Susceptibility Factors As a Driving Force

Besides the unique calcium handling and morphology of nigrostriatal neurons, other susceptibility factors have been proposed. One of the important contributors is  $\alpha$ -synuclein (ASYN). ASYN has become recognized over the past two decades as a key player in PD pathogenetic processes [85–87]. The protein is expressed at high levels in normal brains, is a primary constituent of Lewy bodies (the intraneuronal inclusions found in most PD brains), and can spread in a prion-like pathology across cells [85,88]. Single-point mutations, as well as multiplications of the gene encoding ASYN (*SNCA*), are causally linked to familial PD. Besides the genetic associations of PD and ASYN, there is also an intriguing relationship between ASYN and the outcome of PD-relevant toxic exposures, such as to MPTP. Vila and colleagues were

the first to note that administration of MPTP to mice induced an upregulation of ASYN within nigrostriatal dopaminergic neurons [89], a finding confirmed by subsequent studies in nonhuman primate brains [90]. It is noteworthy that enhancement of intraneuronal ASYN concentrations, even when transitory, could trigger pathological processes such as the formation of aggregated ASYN and interneuronal ASYN transmission [91,92]. Thus, findings in the MPTP model are consistent with the possibility that toxic exposures contribute to PD pathogenesis by modifying ASYN proteostasis. Accordingly, reduced ASYN expression led to resistance to MPTP and rotenone [93,94], whereas overexpression resulted in sensitization [95]. Notably, ASYN level alone cannot explain the relative sensitivities of various catecholaminergic neurons; several brain areas that are highly resistant to MPTP express ASYN at similar levels as nigrostriatal neurons, and DA neurons of the (MPP<sup>+</sup>-resistant) VTA express higher levels of ASYN than do (MPP<sup>+</sup>-sensitive) noradrenergic neurons of the locus coeruleus [96]. Other key variables besides expression also affect ASYN neurotoxicity, including turnover, aggregation state, intraneuronal localization, and post-translational modifications. ASYN phosphorylation and nitration are typically seen in postmortem PD brains [88,97] or in animals treated with MPTP [98], while modified ASYN is not detectable in the nigrostriatal system of untreated animals or healthy humans. Modified and/or augmented ASYN is likely to affect neuronal susceptibility to MPTP, and it might do so in concert with other factors, such as neuronal DA content and Ca<sup>2+</sup>-channel expression [99,100]. ASYN enhances cytosolic DA levels by interfering with DA sequestration [101], and interaction of ASYN with DA potently increases neurotoxicity [102]. These observations not only underscore the relationship between MPTP and ASYN, but also illustrate the concept that specific susceptibility factors might separately be insufficient to cause selective neuronal damage, while a combination of these factors could produce additive or synergistic effects that ultimately lead to the demise of defined neuronal subpopulations [99].

### Concluding Remarks

When precisely nigrostriatal DA neurons start dying in PD is unknown, but PD progression, which takes years, is likely driven by a vicious cycle of events, including mitochondrial function disturbances, cellular proteostasis breakdown, increased oxidative stress, and neuroinflammation [88]. Conversely, a defined molecular initiating event (complex I inhibition) (Figure 3) is recognized in MPTP-induced neurodegeneration, characterized by cell death that is completed within a few days. Despite these differences, this MPTP model involves toxic vicious cycles that might recapitulate neurodegenerative mechanisms relevant to the human disease. To understand the extent to which these similarities and differences between the MPTP model and PD are relevant for pharmacological predictions, it is critical to not only obtain a complete overview of the pathomechanisms, but also integrate the latest observations on MPTP bioactivation and distribution. This review was designed to update the reader on information that substantially departs from current textbook knowledge regarding astrocytic MPP<sup>+</sup> generation and DA neuron degeneration following MPP<sup>+</sup> uptake. The importance of such considerations for therapeutic research is indicated by the frequent use of MPTP models in not only mechanistic investigations, but also pharmacological intervention studies. The MPTP model has been instrumental in the development of synthetic DA receptor agonists, deep-brain stimulation, DA augmentation approaches, and several novel intervention strategies currently in clinical trials [19,22]. The design of a new generation of therapeutic strategies depends on detailed insights into the key events that drive the demise of DA neurons, and on factors, such as the MPTP administration protocol applied, that control such events [103,104]. Systemic integration of available literature data into a framework of molecular key events and their respective relationships is currently being pursued according to the concept of adverse outcome pathways [105,106] (Figure 6). The growing body of identified endogenous susceptibility and resilience factors, together with enhanced knowledge regarding how PD-associated molecular events are reflected by the MPTP model, will allow the development of highly predictive human

### Outstanding Questions

In MPTP models, what is the relative contribution of distinct cell death mechanisms (mitochondrial energy-production impairment, ROS formation, etc.)? Which mechanism might most efficiently be counteracted to achieve neuroprotection?

Are cell death mechanisms and pathological features similar in acute versus chronic MPTP exposure paradigms? How are single neurons affected by acute and/or chronic MPTP exposure?

Neurodegeneration is complete in a few days or weeks in MPTP models, but nigrostriatal degeneration progresses over years in PD. Does degeneration speed differ at the single cell level? Would chronic MPTP-exposure paradigms closely mimic PD pathogenetic changes?

Nigrostriatal degeneration is the main, but not the only pathological feature of MPTP models. Thus, extranigral MPTP pathology must be examined to answer key questions such as: how much does degeneration of other neuronal populations contribute to MPTP-induced behavioral phenotypes?

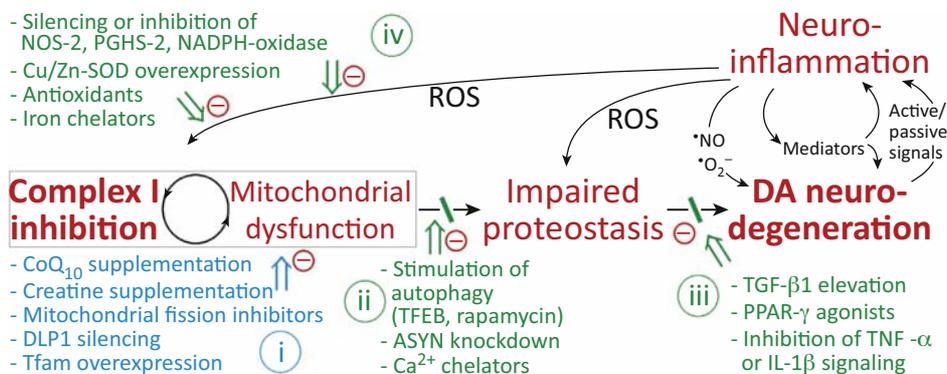
To what extent are behavioral parameters assessed in mice (motor activity, pole test, etc.) representative of parkinsonian motor deficits (e.g., bradykinesia)?

What relative roles do various neuroinflammation mechanisms have in acute and/or subacute MPTP treatment-induced nigrostriatal degeneration?

Aging might affect MPTP toxicity in mice by upregulating MAO-B and, thus, MPP<sup>+</sup> production. Do the same aging-related changes increase nigrostriatal susceptibility to PD pathogenesis and affect outcome in MPTP models?

How does the growing panel of PD-associated proteins (ASYN, PINK1, LRRK2, etc.) contribute to the events leading to selective tissue injury and neurodegeneration in MPTP models? Are the roles of the proteins in MPTP models relevant to our understanding of their roles in idiopathic PD?

Which specific perturbations of proteostasis link mitochondrial inhibition



to the downstream consequence of MPTP exposure (MPP<sup>+</sup> neurotoxicity), and what functions do ASYN and other PD-related proteins perform in this key event?

#### Trends in Pharmacological Sciences

Figure 6. Protection against 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) Toxicity. The concept of adverse outcome pathways (AOPs) has been introduced to organize the vast array of information on toxicity pathways. MPTP is the archetypical example toxicant in a recently assembled AOP linking the inhibition of mitochondrial complex I with parkinsonian motor deficits. A modified version (including feedback loops) is depicted herein to illustrate known intervention strategies. (i) Several strategies interfere with the first steps of the pathogenic cascade. Given that the MPTP model is used to identify pharmacological treatments that could halt neurodegeneration in PD, intervention strategies that target downstream events of the AOP are of particular interest. Such strategies might prevent the adverse outcome despite not targeting either disease initiation or initial damage. (ii) One group of interventions blocks disturbances of cellular proteostasis downstream of mitochondrial dysfunction. (iii) A second group targets the self-amplifying cycle of neuronal damage and neuroinflammation. This might be achieved by the blocking of proinflammatory mediators or their signaling (e.g., IL-1β), or by the augmentation of anti-inflammatory conditions (e.g., by elevation of TGF-β1 or through PPAR-γ agonists). (iv) A final target is reactive oxygen species (ROS), derived from, for example, NADPH oxidase, as well as reactive nitrogen species [e.g., from inducible nitric oxide synthase (NOS-2)]. Alternatively, the cellular antioxidative capacity (Cu/Zn-SOD overexpression) might be augmented. Notably, oxidative stress might be involved both in the transition of mitochondrial dysfunction to impaired proteostasis, and in several enhancing feedback loops (e.g., complex I inhibition through reversible S-nitrosation or irreversible nitration). Abbreviations: ASYN, alpha synuclein; CoQ<sub>10</sub>, coenzyme Q<sub>10</sub>; Cu/Zn-SOD, Cu/Zn-superoxide dismutase; DLP-1, dynamin-like protein-1; NOS-2, nitric oxide synthase-2; PGHS-2, prostaglandin endoperoxide H2 synthase-2; PPAR, peroxisome proliferator activated receptor; Tfam, transcription factor A, mitochondrial; TFEB, transcription factor EB; TGF-β1, transforming growth factor β1.

cell-based *in vitro* PD models, and will also provide the framework for developing entirely novel types of PD intervention.

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