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The importance of choosing a preclinical model that reflects what happens in Parkinson's disease

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A B S T R A C T

One of the major problems in the translation of successful preclinical results to clinical studies and new therapies in Parkinson's disease is the use of preclinical models based on exogenous neurotoxins that do not replicate what happens in the disease. The loss of dopaminergic neurons containing neuromelanin in Parkinson´s disease takes years, contrasting the very rapid degeneration induced by exogenous neurotoxins. We discuss the role of endogenous neurotoxins generated during dopamine oxidation and its possible use as new preclinical models for Parkinson´s disease.

Recently, a paper was published entitled "Mitochondrial alterations in human samples and cellular models of Parkinson's disease", whose aim was to verify the molecular basis of altered mitochondrial dynamics observed in samples of the substantia nigra of sporadic Parkinson's disease patients in the cell line SHSY5Y (undifferentiated) by using 1-methyl-4-phenylpyridinium (MPP+) or dopamine as preclinical toxins (Zilocchi et al., 2018). The protein level of voltage-dependent anion channels (VDACs) decreased in the samples of patients with Parkinson's disease, in comparison with control samples, as well as decreased in SH-SY5Y cells treated with dopamine, while increasing in SH-SY5Y cells treated with MPP+. The protein level of the cytochrome *c* oxidase subunit 5β (COX5β) did not change in patients' samples or in SH-SY5Y cells treated with dopamine, contrasting with a decrease in cells treated with MPP+. The results for cells treated with dopamine better showed the molecular picture observed in the samples of patients with Parkinson's disease. The conclusions of this paper are highly significant to the Parkinson's disease field because preclinical models, based on exogenous neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine and rotenone, are still used to study mechanisms or test new drugs for Parkinson's disease. This is despite the fact that there is a long list of failed clinical studies based on successful preclinical studies performed with exogenous neurotoxins (Athauda and Foltynie, 2015; Lindholm et al., 2015; Park and Stacy, 2015; Olanow et al., 2015a; b). The exogenous neurotoxin MPTP or its metabolite MPP + induces an extremely rapid degeneration, which contrasts with what happen in Parkinson's disease. MPTP induced a severe Parkinsonism in just three days in humans after the use of a synthetic drug contaminated with MPTP (Williams, 1986). The degenerative process of the nigrostriatal neurons, before the appearance of motor symptoms, and the progression of the disease take years (Braak et al., 2004). This explains why MPTP or other exogenous neurotoxins cannot be used to study molecular mechanisms of degeneration or design new disease-modifying drugs for Parkinson's disease (Athauda and Foltynie, 2015). The extremely slow loss of dopaminergic neurons containing neuromelanin in the nigrostriatal system suggests that an endogenous neurotoxin must trigger different mechanisms involved in the degenerative process observed in Parkinson's disease, such as mitochondrial dysfunction, alpha-synuclein aggregation to neurotoxic oligomers, protein degradation dysfunction of both lysosomal and proteasomal systems, endoplasmic reticulum stress, neuroinflammation and oxidative stress. It seems plausible that the endogenous neurotoxin, which triggers all these mechanisms involved in the loss of dopaminergic neurons containing neuromelanin in the nigrostriatal system, is formed during dopamine oxidation.

Zilocchi et al. (2018) paper showed that the results obtained with MPP+ were different to those obtained with dopamine alone, which more closely resembles what was observed in Parkinson's patients. VDAC1 and 2 were reduced by dopamine but increased by MPP+. COX5β was found unchanged by dopamine, while it was lowered by MPP+. Dopamine toxicity *in vivo* has been demonstrated to produce both pre- and postsynaptic damage to nigrostriatal structures, suggesting that dopamine could act as a low-potency neurotoxin (Filloux and Townsend, 1993). Intrastriatal injection of dopamine induces selective reduction of tyrosine hydroxylase staining, with a concomitant increase in indices of dopamine oxidation. The specific loss of tyrosine hydroxylase immunoreactivity was no longer detectable after the injection of dopamine with an equimolar concentration of either ascorbic acid or glutathione (Hastings et al., 1996, Rabinovic et al., 2000). Changing both dopamine levels and alpha-synuclein expression in aged mice induces progressive neurodegeneration (Mor et al., 2019). Free cytosolic dopamine induces neurodegeneration in transgenic mice that selectively overexpress the dopamine transporter in dopamine neurons.

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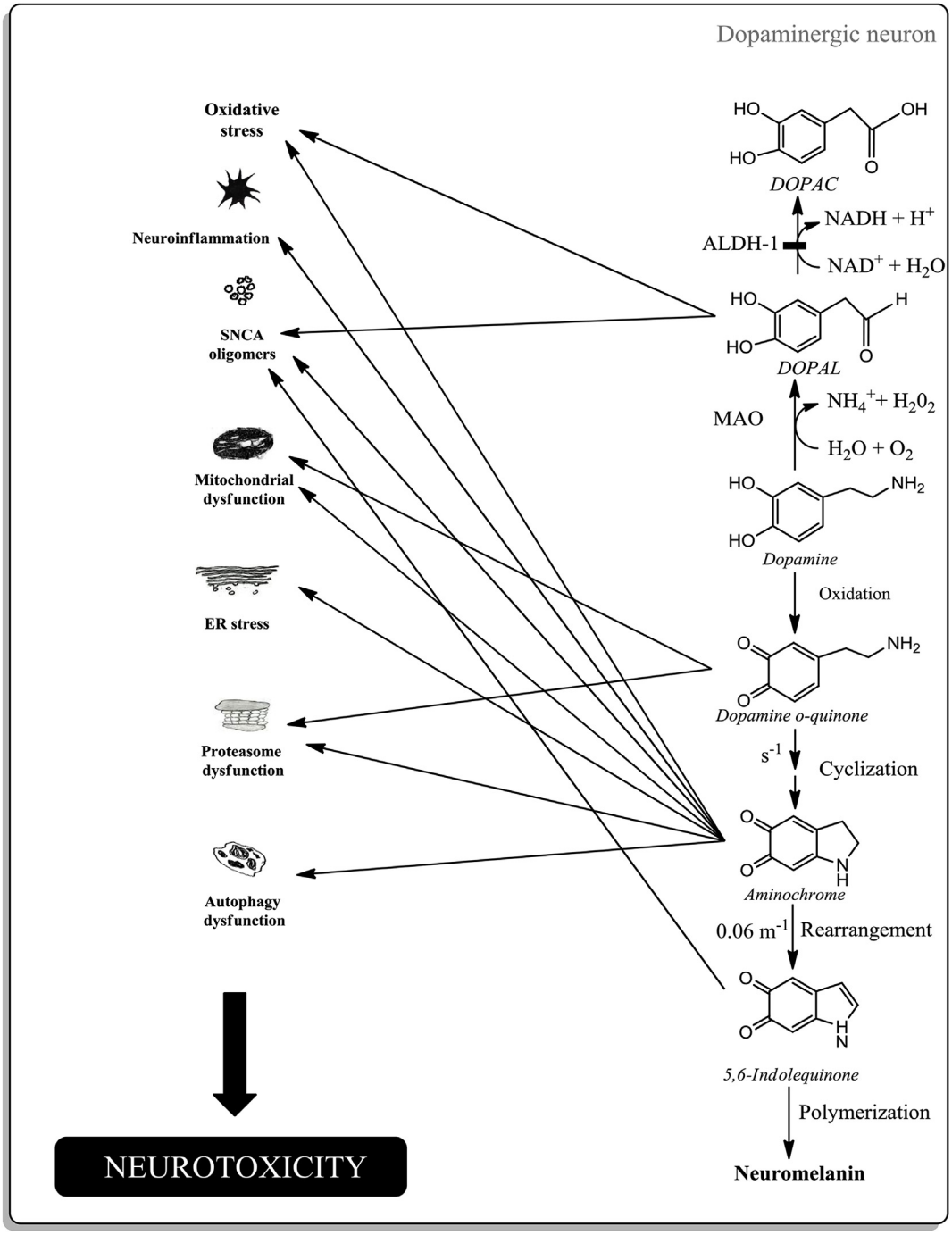

Fig. 1. Neurotoxic reactions during dopamine oxidation. Cytosolic dopamine undergoes oxidative deamination catalyzed by monoamine oxidase (MAO) to 3,4-dihydroxyphenylacetaldehyde (DOPAL), with the concomitant formation of ammonia and hydrogen peroxide. DOPAL is converted to DOPAC, which is catalyzed by aldehyde dehydrogenase-1 and 2. However, aldehyde dehydrogenase-1 (ALDH-1) has lower activity in Parkinson's patients, explaining DOPAL accumulation and neurotoxic effects. DOPAL induces oxidative stress and alpha-synuclein aggregation. Dopamine *o*-quinone, the first intermediate formed during dopamine oxidation to neuromelanin, induces proteasome and mitochondrial dysfunction. 5,6-indolequinone is demonstrated by NMR reacting with alpha-synuclein. Aminochrome induces oxidative stress, neuroinflammation, alpha-synuclein aggregation to neurotoxic oligomers, mitochondrial dysfunction, endoplasmic reticulum stress, and proteasome and autophagy dysfunction.

These mice experience a spontaneous loss of midbrain dopamine neurons and increased oxidative stress markers, 5-S-cysteinyl-dopamine and 5-S-cysteinyl-DOPAC (Masoud et al., 2015). Motor dysfunctions, progressive neurodegeneration in the striatum, a substantial decrease in glutathione, and oxidative protein modifications were observed in a transgenic mouse with the ability to take up extracellular dopamine without acquiring regulatory mechanisms found in dopamine neurons (Chen et al., 2008). Dopamine toxicity seems to be dependent on its ability to generate neurotoxic metabolites during its oxidation (Fig. 1).

Dopamine is synthetized in dopaminergic neurons and stored in monoaminergic vesicles for neurotransmission, where it is completely stable; in the cytosol, however, free dopamine can oxidize:

(i) Dopamine can undergo oxidative deamination, catalyzed by monoamine oxidase, into 3,4-dihydroxyphenylacetaldehyde (DOPAL). Aldehyde dehydrogenase catalyzes the conversion of

DOPAL into 3,4-dihydroxyphenylacetic acid. There are two forms of aldehyde dehydrogenase, i.e., −1 and −2; only aldehyde dehydrogenase-1 is low expressed in Parkinson's disease patients, resulting in the accumulation of DOPAL, which cannot be degraded to DOPAC. Mitochondrial aldehyde dehydrogenase-2 was significantly increased in the putamen of patients compared to controls. (Grünblatt et al., 2004, 2018; Grünblatt and Riederer, 2016; Mandel et al., 2005; Goldstein et al., 2012, 2014; Deza-Ponzio et al., 2018; Michel et al., 2014). Intranigral injection of DOPAL induces the loss of tyrosine hydroxylase positive staining (Burke et al., 2003; Panneton et al., 2010). DOPAL oxidation gives rise to a transient DOPAL semiquinone radical and an ortho-quinone, which exists exclusively in hydrated form (Anderson et al., 2011). N-acetylcysteine decreases DOPAL-quinone formation and spontaneous dopamine oxidation during MAO inhibition (Jinsmaa et al., 2018; Goldstein et al., 2017). DOPAL covalently modifies and inhibits tyrosine hydroxylase (Mexas et al., 2011). DOPAL induces mitochondrial dysfunction (Kristal et al., 2001). DOPAL also induces alpha-synuclein aggregation, which is enhanced by divalent metal ions (Jinsmaa et al., 2014, 2018; Burke et al., 2008; Werner-Allen et al., 2016, 2017; 2018; Follmer et al., 2015; Plotegher et al., 2017). Alpha-synuclein mutations increase the formation of DOPAL-induced alpha-synuclein oligomers (Lima et al., 2018). Methionine oxidation of C-terminal domain alpha-synuclein is involved in the formation of large oligomers induced by DOPAL (Coelho-Cerqueira et al., 2019; Carmo-Gonçalves et al., 2018). Alpha-synuclein binds to monoamine oxidase-B (MAO-B), stimulating its enzymatic activity, which triggers asparagine endopeptidase activation and subsequent alpha-synuclein cleavage at N103. Rasagiline, an inhibitor of MAO-B decreases alpha-synuclein-induced pathology and motor dysfunction (Kang et al., 2018). Adducts formed between DOPAL and aminoindan or rasagiline were detected by mass spectrometry, with both compounds exerting a neuroprotective effect against DOPAL-induced toxicity in cells (Kumar et al., 2019);

(ii) Dopamine oxidation into neuromelanin involves the formation of several ortho(*o*)-quinones, such as dopamine *o*-quinone, aminochrome and 5,6-indolequinone (Fig. 1). Under certain conditions, these *o*-quinones can be neurotoxic (Fig. 1). Dopamine *o*-quinone has been reported to form adducts with several mitochondrial proteins, such as mitochondrial creatine kinase, ubiquinol-cytochrome c reductase core protein 1, ubiquitin carboxy-terminal hydrolase L1 (UCHL-1), glucose-regulated protein 75/mitochondrial HSP70/mortalin, mitofilin, glutathione peroxidase-4 and cytosolic proteins, such as Parkinsonism-associated deglycase (DJ-1, PARK7), and human dopamine transporter, alpha synuclein and parkin (Van Laar et al., 2009; Hauser et al., 2013; Whitehead et al., 2001; LaVoie et al., 2005; Conway et al., 2001). However, dopamine *o*-quinone is unstable at physiological pH and immediately cyclizes into aminochrome at a rate of s^{-1} (Tse et al., 1976; Segura-Aguilar and Lind, 1989). Aminochrome is the most “stable” and studied of the *o*-quinones formed during neuromelanin synthesis. Aminochrome is rendered neurotoxic by inducing mitochondrial dysfunction (Aguirre et al., 2012; Arriagada et al., 2004; Paris et al., 2011; Muñoz et al., 2012a; Huenchuguala et al., 2017; Segura-Aguilar and Huenchuguala, 2018), proteasome dysfunction (Zafar et al., 2006; Zhou and Lim, 2009), oxidative stress (Arriagada et al., 2004), autophagy dysfunction (Huenchuguala et al., 2014; Muñoz et al., 2012b), the disruption of cytoskeleton architecture (Paris et al., 2010), the inhibition of microtubule polymerization (Briceño et al., 2016), neuroinflammation (de Araújo et al., 2018; Santos et al., 2017), lysosome dysfunction (Meléndez et al., 2019), endoplasmic reticulum stress (Xiong et al., 2014), and the formation of neurotoxic oligomers of alpha-synuclein (Muñoz et al., 2015; Muñoz and Segura-Aguilar, 2017). Aminochrome is rearranged into 5,6-indolequinone at a rate of 0.06 min^{-1} (Bisaglia et al., 2007), while it has been reported that 5,6-indolequinone forms adducts with alpha-synuclein (Bisaglia et al., 2007). A non-identified *o*-quinone called dopaminochrome, which forms during dopamine oxidation, has been reported but its structure remains unknown. Dopaminochrome is not aminochrome because its absorption spectrum is different (dopaminochrome has a peak at 303 and 479 nm, while aminochrome has a peak at 287, and 477 nm), and aminochrome structure has been determined by NMR (Ochs et al., 2005; Paris et al., 2010). It seems plausible that it corresponds to 5,6-indolequinone. Dopaminochrome has been reported to be neurotoxic in cell cultures (Linsenbardt et al., 2009, 2012), while the unilateral injection of dopaminochrome induced a slow and progressive degeneration in the dopaminergic neurons in the substantia nigra (Touchette et al., 2016). Dopaminochrome induces reversible inhibition of alpha-synuclein fibrillization by promoting conformational changes resulting in the formation of spherical oligomers. Dopaminochrome induces conformational changes by interacting with the 125YEMPS129 motif of alpha-synuclein (Norris and Giasson, 2005).

Dopamine oxidation into neuromelanin is a normal and harmless pathway because neuromelanin accumulates with age, with healthy seniors having intact dopaminergic neurons in their substantia nigra (Zecca et al., 2002). It is a paradox that neurotoxic *o*-quinones are formed during the synthesis of neuromelanin, which is a harmless process. An explanation for this paradox is the existence of two enzymes, which prevent aminochrome neurotoxicity (Fig. 2). DT-diaphorase, a flavoenzyme expressed in dopaminergic neurons and astrocytes, catalyzes the two-electron reduction of aminochrome into leukoaminochrome (Segura-Aguilar and Lind, 1989), preventing aminochrome-induced cell death (Lozano et al., 2010), mitochondrial dysfunction (Arriagada et al., 2004; Muñoz et al., 2012a; Paris et al., 2011), lysosome and autophagy dysfunction (Meléndez et al., 2019; Muñoz et al., 2012b; Huenchuguala et al., 2014), proteasome dysfunction (Zafar et al., 2006), the disruption of cytoskeleton architecture (Paris et al., 2010), oxidative stress (Arriagada et al., 2004), and the formation of neurotoxic alpha-synuclein oligomers (Muñoz et al., 2015; Muñoz and Segura-Aguilar, 2017). Glutathione transferase M2-2 (GSTM2), expressed in human astrocytes, catalyzes the GSH conjugation of aminochrome into 4-S-glutathionyl-5,6-dihydroxyindoline, which does not oxidize in the presence of superoxide, oxygen and hydrogen peroxide (Segura-Aguilar et al., 1997; Baez et al., 1997). GSTM2 also catalyzes the GSH conjugation of the aminochrome precursor dopamine *o*-quinone into 5-glutathionyl-dopamine (Dagnino-Subiabre et al., 2000). 5-glutathionyl-dopamine is enzymatically degraded to 5-cysteinyl dopamine, which is an end metabolite that can be eliminated from the cells. Interestingly, 5-cysteinyl-dopamine has been detected in the substantia nigra, globus pallidus, putamen, caudate nucleus, neuromelanin and cerebrospinal fluid of Parkinson's disease patients (Carstam et al., 1991; Rosengren et al., 1985; Cheng et al., 1996). GSTM2 prevents aminochrome-induced neurotoxicity, autophagy, lysosome dysfunction and mitochondrial dysfunction (Huenchuguala et al., 2014, 2017; Segura-Aguilar and Huenchuguala, 2018). Aminochrome, conjugated with glutathione, which is catalyzed by GSTM2 in the presence of alpha-synuclein, forms nontoxic alpha-synuclein oligomers (Huenchuguala et al., 2019).

The protective role of GSTM2 is not restricted to astrocytes, where it is expressed, as this enzyme also protects dopaminergic neurons by secreting GSTM2 from astrocytes, which dopaminergic neurons internalize into the cytosol to prevent aminochrome-induced neurotoxicity (Cuevas et al., 2015). The question is why DT-diaphorase and GSTM2 do not protect dopaminergic neurons containing neuromelanin against aminochrome neurotoxicity in Parkinson's patients. A possible explanation for the lack of protection against aminochrome neurotoxicity is that (i) aminochrome concentration surpasses protective enzymes' capacity as a consequence of a localized increase in free dopamine in a

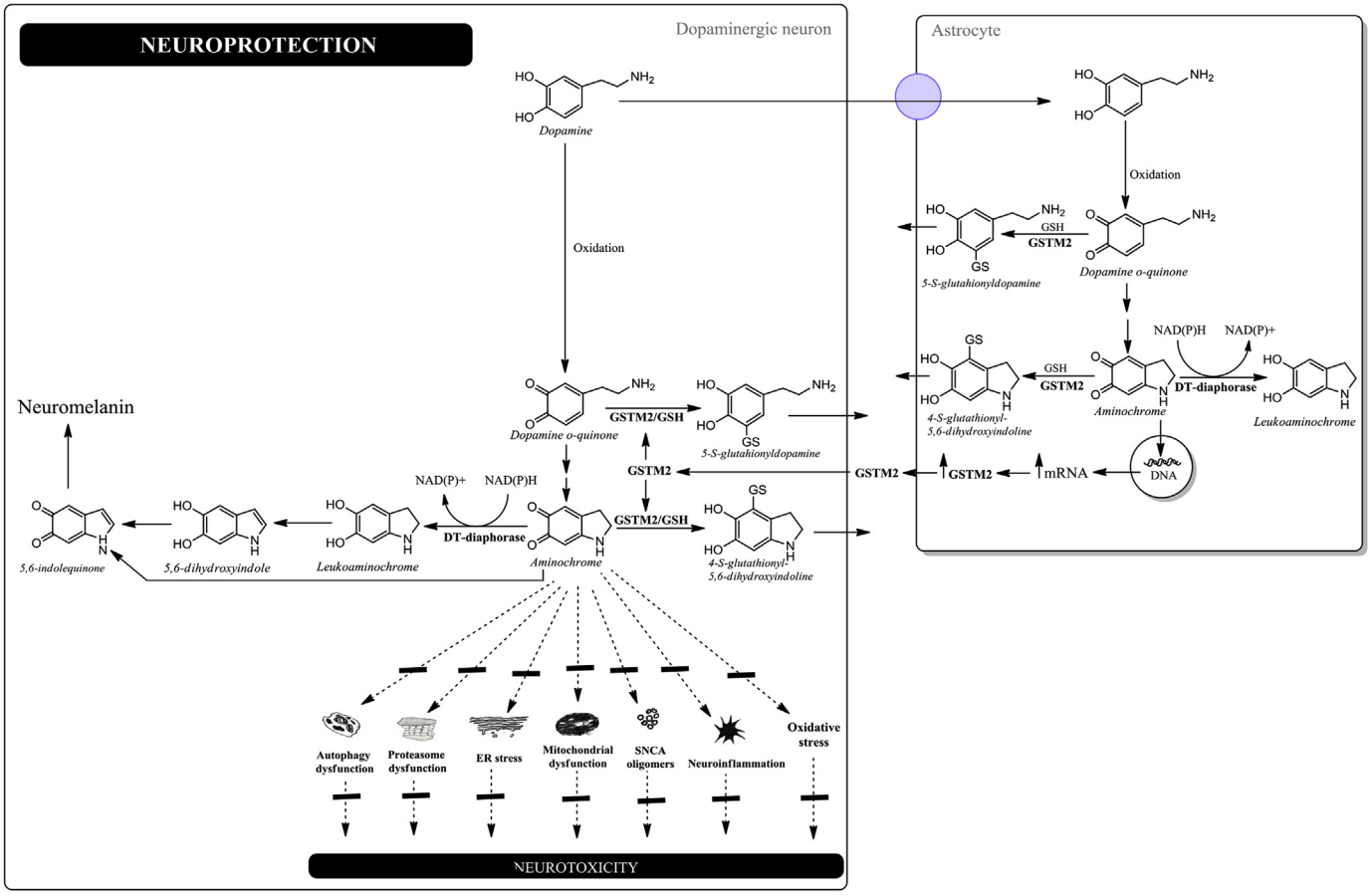

Fig. 2. Neuroprotective reactions in dopamine oxidation to aminochrome. Dopamine oxidizes into dopamine *o*-quinone, which immediately cyclizes into aminochrome. Aminochrome can be neurotoxic by inducing autophagy and proteasome dysfunction, endoplasmic reticulum stress, mitochondrial dysfunction, the formation of alpha-synuclein (SNCA) neurotoxic oligomers, neuroinflammation and oxidative stress. However, DT-diaphorase and GSTM2 prevent aminochrome toxicity, in both dopaminergic neurons and astrocytes. GSTM2 is expressed in astrocytes, but this enzyme also protects dopaminergic neurons by secreting GSTM2, which dopaminergic neurons are internalized in the cytosol to prevent aminochrome-induced neurotoxicity.

single dopaminergic neuron, leading to an increase of dopamine oxidation to *o*-quinones, such as aminochrome. Vesicular monoamine transporter-2 (VMAT-2) plays a key role in the prevention of dopamine oxidation since it transports dopamine into monoaminergic vesicles, where dopamine is completely stable and accumulates for neurotransmission. Dopamine transport into monoaminergic vesicles, mediated by VMAT-2, is coupled to an ATPase, which pumps protons into the vesicle, decreasing its pH (Segura-Aguilar et al., 2014). The existence of a kind of complex of tyrosine hydroxylase, aromatic L-amino acid decarboxylase and VMAT-2 prevents the release of free cytosolic dopamine during dopamine synthesis from tyrosine, which is localized in the membrane of monoaminergic vesicles (Cartier et al., 2010). Neuromelanin formation is dependent on dopamine oxidation to *o*-quinones; therefore, the level of VMAT-2 expression is inversely correlated with the level of neuromelanin in dopaminergic neurons (Liang et al., 2004). Overexpression of VMAT-2 prevents neuromelanin formation (Sulzer et al., 2000). Dopamine uptake and binding of VMAT-2 inhibitor were significantly decreased in monoaminergic vesicles isolated from Parkinson's disease patients' brains, in comparison to control brains (Pifl et al., 2014). It seems to be plausible that, under certain conditions, VMAT-2 expression is downregulated or inhibited, allowing the existence of free dopamine in excess surpassing neuroprotective enzyme capacity, leading to the death of a single neuron. Over time (years), the loss of a single dopaminergic neuron containing neuromelanin accumulate until the motor symptoms appear, and (ii) the expression level of DT-diaphorase and GSTM2 decreases, or they are inhibited under certain unknown conditions, allowing the existence of no metabolized aminochrome. Lack of metabolism by protective enzymes may lead to one-electron reduction of aminochrome into leukoaminochrome *o*-semiquinone radical, generating redox cycling between this radical, which autoxidizes by reducing oxygen to superoxide and aminochrome. This redox cycling depletes NADH and oxygen, in turn inducing mitochondrial dysfunction, oxidative stress and neuroinflammation, and/or form adducts with proteins such as alpha-synuclein, complex I, proteasome, endoplasmic reticulum, vacuolar H-type ATPase localized in lysosome membrane, tubulin and actin.

Zilocchi et al. (2018) paper used two different neurotoxin models in SH-SY5Y cells, dopamine and the complex I inhibitor MPP+, to verify the molecular basis of altered mitochondrial dynamics observed in samples of the substantia nigra of sporadic Parkinson's disease patients. MPP+ is the neurotoxic metabolite of the exogenous neurotoxin MPTP. The major disadvantages of MPP + as a neurotoxin model are as follows:

(i) It is an exogenous neurotoxin and induces acute degeneration, in contrast with the extremely slow degeneration observed in the disease. MPP + has a high affinity to dopamine transporter and affects all possible dopaminergic neurons expressing dopamine transporter, resulting in severe Parkinsonism in just three days (Williams, 1986).
(ii) Results from preclinical studies cannot be translated to successful clinical studies (Athauda and Foltynie, 2015).
(iii) MPP+ is a complex I inhibitor, inducing mitochondrial dysfunction (Kim et al., 2018; Zhao et al., 2019; Liu et al., 2017), while

ATP depletion seems to be the major cause of MPP + -induced dopamine neuronal death (Wang et al., 2007).

It is, in general, believed that autophagy dysfunction plays an important role in the degeneration of nigrostriatal neurons in Parkinson's disease (Karabiyik et al., 2017), while autophagy (mitophagy) plays a highly important role in maintaining mitochondrial function (Huenchuguala et al., 2017). However, MPP + has been reported to induce autophagy over activation (Nopparat et al., 2014; Zhu et al., 2007; Xie et al., 2019; Zhao et al., 2019), but mitochondrial dysfunction was not restored. There is a limited body of literature on dopamine toxicity *in vivo*; but, in general, it is accepted that the neurotoxic action of dopamine depends on dopamine's ability to oxidize to DOPAL and *o*-quinones, such as aminochrome, during neuromelanin synthesis. The neurotoxicity of DOPAL is based on the low mRNA expression of aldehyde dehydrogenase-1 in postmortem samples of Parkinson's disease patients (Galter et al., 2003). It is unclear how aldehyde dehydrogenase-1 is downregulated in Parkinson's disease, and there are several ways to repress or induce the expression of a gene in a cell. In postmortem tissue, total RNA is extracted for the nucleotide array (Grünblatt et al., 2004) and probably changes in the promotor sequence affecting RNA polymerase interaction, with the promoter resulting in changes in the rate of DNA transcription. Modification of the promotor sequence will probably result in the early onset of the disease in a familial form, while its relevance in the sporadic form of the disease is clear. Dopamine oxidation to neuromelanin generates aminochrome inside of the dopaminergic neurons containing neuromelanin, which are lost in the disease, and triggers all the mechanisms proposed to be involved in the degeneration of the nigrostriatal system.

In conclusion, the relevance of Zilocchi et al. (2018) paper is that it: (i) supports the idea that exogenous neurotoxins are not suitable for testing new drugs or studying mechanisms for Parkinson's disease (which could come as a surprise to many scientists who are still using these preclinical models); and (ii) demonstrates that preclinical models based on dopamine are closer to what happens in the disease.

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Appendix A. Supplementary data

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References

Aguirre, P., Urrutia, P., Tapia, V., Villa, M., Paris, I., Segura-Aguilar, J., Nuñez, M.T., 2012. The dopamine metabolite aminochrome inhibits mitochondrial complex I and modifies the expression of iron transporters DMT1 and FPN1. Biometals 25, 795–803.

Anderson, D.G., Mariappan, S.V., Buettner, G.R., Doorn, J.A., 2011. Oxidation of 3,4-dihydroxyphenylacetaldehyde, a toxic dopaminergic metabolite, to a semiquinone radical and an ortho-quinone. J. Biol. Chem. 286, 26978–26986. https://doi.org/10.1074/jbc.M111.249532.

Arriagada, C., Paris, I., Sanchez de las Matas, M.J., Martinez-Alvarado, P., Cardenas, S., Castañeda, P., et al., 2004. On the neurotoxicity mechanism of leukoaminochrome o-semiquinone radical derived from dopamine oxidation: mitochondria damage, necrosis, and hydroxyl radical formation. Neurobiol. Dis. 16, 468–477.

Athauda, D., Foltynie, T., 2015. The ongoing pursuit of neuroprotective therapies in Parkinson disease. Nat. Rev. Neurol. 11, 25–40.

Baez, S., Segura-Aguilar, J., Widersten, M., Johansson, A.S., Mannervik, B., 1997. Glutathione transferases catalyse the detoxication of oxidized metabolites (o-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes. Biochem. J. 324 (Pt 1), 25–28.

Bisaglia, M., Mammi, S., Bubacco, L., 2007. Kinetic and structural analysis of the early oxidation products of dopamine: analysis of the interactions with alpha-synuclein. J. Biol. Chem. 282, 15597–15605.

Braak, H., Ghebremedhin, E., et al., 2004. Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res. 318, 121–134.

Briceño, A., Muñoz, P., Brito, P., Huenchuguala, S., Segura-Aguilar, J., Paris, I.B., 2016. Aminochrome toxicity is mediated by inhibition of microtubules polymerization through the formation of adducts with tubulin. Neorotox. Res. 29, 381–393.

Burke, W.J., Kumar, V.B., Pandey, N., Panneton, W.M., Gan, Q., Franko, M.W., et al., 2008. Aggregation of alpha-synuclein by DOPAL, the monoamine oxidase metabolite of dopamine. Acta Neuropathol. 115, 193–203.

Burke, W.J., Li, S.W., Williams, E.A., Nonneman, R., Zahm, D.S., 2003. 3,4-Dihydroxyphenylacetaldehyde is the toxic dopamine metabolite in vivo: implications for Parkinson's disease pathogenesis. Brain Res. 989, 205–213.

Carmo-Gonçalves, P., do Nascimento, L.A., Cortines, J.R., Eliezer, D., Romão, L., Follmer, C., 2018. Exploring the role of methionine residues on the oligomerization and neurotoxic properties of DOPAL-modified α-synuclein. Biochem. Biophys. Res. Commun. 505, 295–301. https://doi.org/10.1016/j.bbrc.2018.09.111. Epub 2018 Sep 22.

Carstam, R., Brinck, C., et al., 1991. The neuromelanin of the human substantia nigra. Biochim. Biophys. Acta 1097, 152–160.

Cartier, E.A., Parra, L.A., Baust, T.B., Quiroz, M., Salazar, G., Faundez, V., Egaña, L., Torres, G.E., 2010. A biochemical and functional protein complex involving dopamine synthesis and transport into synaptic vesicles. J. Biol. Chem. 151https://doi.org/10.1074/jbc.M109.054510. 957-666.

Chen, L., Ding, Y., Cagniard, B., Van Laar, A.D., Mortimer, A., Chi, W., Hastings, T.G., Kang, U.J., Zhuang, X., 2008. Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. J. Neurosci. 28, 425–433.

Cheng, F.C., Kuo, J.S., et al., 1996. Elevated 5-S-cysteinyldopamine/homovanillic acid ratio and reduced homovanillic acid in cerebrospinal fluid. J. Neural Transm. 103, 433–446.

Coelho-Cerqueira, E., de Araújo Correia Campos, C., Follmer, C., 2019. Formation of large oligomers of DOPAL-modified α-synuclein is modulated by the oxidation of methionine residues located at C-terminal domain. Biochem. Biophys. Res. Commun. 509, 367–372. https://doi.org/10.1016/j.bbrc.2018.12.128.

Conway, K.A., Rochet, J.C., Bieganski, R.M., Lansbury Jr., P.T., 2001. Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. Science 294, 1346–1349. https://doi.org/10.1126/science.1063522.

Cuevas, C., Huenchuguala, S., Muñoz, P., Villa, M., Paris, I., Mannervik, B., Segura-Aguilar, J., 2015. Glutathione transferase-M2-2 secreted from glioblastoma cell protects SH-SY5Y cells from aminochrome neurotoxicity. Neurotox. Res. 27, 217–228.

Dagnino-Subiabre, A., Cassels, B.K., Baez, S., Johansson, A.S., Mannervik, B., Segura-Aguilar, J., 2000. Glutathione transferase M2-2 catalyzes conjugation of dopamine and dopa o-quinones. Biochem. Biophys. Res. Commun. 274, 32–36.

de Araújo, F.M., Ferreira, R.S., Souza, C.S., Dos Santos, C.C., Rodrigues, T.L.R.S., e. Silva, J.H.C., Gasparotto, J., Gelain, D.P., El-Bachá, R.S.,D., Costa, M.F., Fonseca, J.C.M., Segura-Aguilar, J., Costa, S.L., Silva, V.D.A., 2018. Aminochrome decreases NGF, GDNF and induces neuroinflammation in organotypic midbrain slice cultures. Neurotoxicology 66, 98–106.

Deza-Ponzio, R., Herrera, M.L., Bellini, M.J., Virgolini, M.B., Hereñú, C.B., 2018. Aldehyde dehydrogenase 2 in the spotlight: the link between mitochondria and neurodegeneration. Neurotoxicology 68, 19–24 2018.

Filloux, F., Townsend, J.J., 1993. Pre- and postsynaptic neurotoxic effects of dopamine demonstrated by intrastriatal injection. Exp. Neurol. 119, 79–88. https://doi.org/10.1006/exnr.1993.1008.

Follmer, C., Coelho-Cerqueira, E., Yatabe-Franco, D.Y., Araujo, G.D., Pinheiro, A.S., Domont, G.B., Eliezer, D., 2015. Oligomerization and membrane-binding properties of covalent adducts formed by the interaction of α-synuclein with the toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL). J. Biol. Chem. 290, 27660–27679.

Galter, D., Buervenich, S., Carmine, A., Anvret, M., Olson, L., 2003. ALDH1 mRNA: presence in human dopamine neurons and decreases in substantia nigra in Parkinson's disease and in the ventral tegmental area in schizophrenia. Neurobiol. Dis. 14, 637–647.

Goldstein, D.S., Jinsmaa, Y., Sullivan, P., Sharabi, Y., 2017. N-acetylcysteine prevents the increase in spontaneous oxidation of dopamine during monoamine oxidase inhibition in PC12 cells. Neurochem. Res. 42, 3289–3295. https://doi.org/10.1007/s11064-017-2371-0.

Goldstein, D.S., Kopin, I.J., Sharabi, Y., 2014. Catecholamine autotoxicity. Implications for pharmacology and therapeutics of Parkinson disease and related disorders. Pharmacol. Ther. 144, 268–282.

Goldstein, D.S., Sullivan, P., Cooney, A., Jinsmaa, Y., Sullivan, R., Gross, D.J., et al., 2012. Vesicular uptake blockade generates the toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde in PC12 cells: relevance to the pathogenesis of Parkinson's disease. J. Neurochem. 123, 932–943.

Grünblatt, E., Mandel, S., Jacob-Hirsch, J., Zeligson, S., Amariglo, N., Rechavi, G., Li, J., Ravid, R., Roggendorf, W., Riederer, P., Youdim, M.B., 2004. Gene expression profiling of parkinsonian substantia nigra pars compacta; alterations in ubiquitin-proteasome, heat shock protein, iron and oxidative stress regulated proteins, cell adhesion/cellular matrix and vesicle trafficking genes. J. Neural Transm. 111, 1543–1573.

Grünblatt, E., Riederer, P., 2016. Aldehyde dehydrogenase (ALDH) in Alzheimer's and Parkinson's disease. J. Neural Transm. 123, 83–90. https://doi.org/10.1007/s00702-014-1320-1.

Grünblatt, E., Ruder, J., Monoranu, C.M., Riederer, P., Youdim, M.B., Mandel, S.A., 2018. Differential alterations in metabolism and proteolysis-related proteins in human Parkinson's disease substantia nigra. Neurotox. Res. 33, 560–568. https://doi.org/10.1007/s12640-017-9843-5.

Hastings, T.G., Lewis, D.A., Zigmond, M.J., 1996. Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. Proc. Natl. Acad. Sci. U. S. A. 93, 1956–1961.

Hauser, D.N., Dukes, A.A., 2013. Dopamine quinone modifies and decreases the abundance of the mitochondrial selenoprotein glutathione peroxidase 4. Free Radic. Biol.

Med. 65, 419–427.
Huenchuguala, S., Muñoz, P., Segura-Aguilar, J., 2017. The importance of mitophagy in maintaining mitochondrial function in U373MG cells. Bafilomycin A1 restores aminochrome-induced mitochondrial damage. ACS Chem. Neurosci. 8, 2247–2253.
Huenchuguala, S., Muñoz, P., Zavala, P., Villa, M., Cuevas, C., Ahumada, U., Graumann, R., Nore, B.F., Couve, E., Mannervik, B., Paris, I., Segura-Aguilar, J., 2014. Glutathione transferase mu 2 protects glioblastoma cells against aminochrome toxicity by preventing autophagy and lysosome dysfunction. Autophagy 10, 618–630.
Huenchuguala, S., Sjödin, B., et al., 2019. Novel alpha-synuclein oligomers formed with the aminochrome-glutathione conjugate are not neurotoxic. Neurotox. Res. 35, 432–440. https://doi.org/10.1007/s12640-018-9969-0.
Jinsmaa, Y., Sharabi, Y., Sullivan, P., Isonaka, R., Goldstein, D.S., 2018. 3,4-Dihydroxyphenylacetaldehyde-Induced protein modifications and their mitigation by N-acetylcysteine. J. Pharmacol. Exp. Ther. 366, 113–124. https://doi.org/10.1124/jpet.118.248492.
Jinsmaa, Y., Sullivan, P., Gross, D., Cooney, A., Sharabi, Y., Goldstein, D.S., 2014. Divalent metal ions enhance DOPAL-induced oligomerization of alpha-synuclein. Neurosci. Lett. 569, 27–32.
Kang, S.S., Ahn, E.,H., Zhang, Z., Liu, X., Manfredsson, F.P., Sandoval, I.M., Dhakal, S., Iuvone, P.M., Cao, X., Ye, K., 2018. α-Synuclein stimulation of monoamine oxidase-B and legumain protease mediates the pathology of Parkinson's disease. EMBO J. 37, e98878. https://doi.org/10.15252/embj.201798878.
Karabiyik, C., Lee, M.J., Rubinsztein, D.C., 2017. Autophagy impairment in Parkinson's disease. Essays Biochem. 61, 711–720. https://doi.org/10.1042/EBC20170023.
Kim, H.Y., Jeon, H., Kim, H., Koo, S., Kim, S., 2018. Sophora flavescens aiton decreases MPP+-Induced mitochondrial dysfunction in SH-SY5Y cells. Front. Aging Neurosci. 10, 119. https://doi.org/10.3389/fnagi.2018.00119.
Kristal, B.S., Conway, A.D., Brown, A.M., Jain, J.C., Ulluci, P.A., Li, S.W., Burke, W.J., 2001. Selective dopaminergic vulnerability: 3,4-dihydroxyphenylacetaldehyde targets mitochondria. Free Radic. Biol. Med. 30, 924–931.
Kumar, V.B., Hsu, F.F., Lakshmi, V.M., Gillespie, K.N., Burke, W.J., 2019. Aldehyde adducts inhibit 3,4-dihydroxyphenylacetaldehyde-induced α-synuclein aggregation and toxicity: implication for Parkinson neuroprotective therapy. Eur. J. Pharmacol. 845, 65–73. https://doi.org/10.1016/j.ejphar.2018.12.027.
LaVoie, M.J., Ostaszewski, B.L., Weihofen, A., Schlossmacher, M.G., Selkoe, D.J., 2005. Dopamine covalently modifies and functionally inactivates parkin. Nat. Med. 11, 1214–1221.
Liang, C.L., Nelson, O., Yazdani, U., Pasbakhsh, P., German, D.C., 2004. Inverse relationship between the contents of neuromelanin pigment and the vesicular monoamine transporter-2: human midbrain dopamine neurons. J. Comp. Neurol. 473, 97–106.
Lima, V.A., do Nascimento, L.A., Eliezer, D., Follmer, C., 2018. Role of Parkinson's disease-linked mutations and N-terminal acetylation on the oligomerization of α-synuclein induced by 3,4-dihydroxyphenylacetaldehyde. ACS Chem. Neurosci. https://doi.org/10.1021/acschemneuro.8b00498. 2018 Nov 5.
Lindholm, D., Mäkelä, J., Di Liberto, V., Mudò, G., Belluardo, N., Eriksson, O., Saarma, M., 2015. Current disease modifying approaches to treat Parkinson's disease. Cell. Mol. Life Sci. 73, 1365–1379.
Linsenbardt, A.J., Breckenridge, J.M., Wilken, G.H., Macarthur, H., 2012. Dopaminochrome induces caspase-independent apoptosis in the mesencephalic cell line, MN9D. J. Neurochem. 122, 175–184.
Linsenbardt, A.J., Wilken, G.H., Westfall, T.C., Macarthur, H., 2009. Cytotoxicity of dopaminochrome in the mesencephalic cell line, MN9D, is dependent upon oxidative stress. Neurotoxicology 30, 1030–1035.
Liu, Y., Chong, L., Li, X., Tang, P., Liu, P., Hou, C., Zhang, X.1., Li, R., 2017. Astragaloside IV rescues MPP+-induced mitochondrial dysfunction through upregulation of methionine sulfoxide reductase A. Exp. Ther. Med. 14, 2650–2656. https://doi.org/10.3892/etm.2017.4834.
Lozano, J., Muñoz, P., Nore, B.F., Ledoux, S., Segura-Aguilar, J., 2010. Stable expression of short interfering RNA for DT-diaphorase induces neurotoxicity. Chem. Res. Toxicol. 23, 1492–1496.
Mandel, S., Grunblatt, E., Riederer, P., Amariglio, N., Jacob-Hirsch, J., Rechavi, G., Youdim, M.B., 2005. Gene expression profiling of sporadic Parkinson's disease substantia nigra pars compacta reveals impairment of ubiquitin-proteasome subunits, SKP1A, aldehyde dehydrogenase, and chaperone HSC-70. Ann. N. Y. Acad. Sci. 1053, 356–375. https://doi.org/10.1196/annals.1344.031.
Masoud, S.T., Vecchio, L.M., Bergeron, Y., Hossain, M.M., Nguyen, L.T., Bermejo, M.K., Kile, B., Sotnikova, T.D., Siesser, W.B., Gainetdinov, R.R., Wightman, R.M., Caron, M.G., Richardson, J.R., Miller, G.W., Ramsey, A.J., Cyr, M., Salahpour, A., 2015. Increased expression of the dopamine transporter leads to loss of dopamine neurons, oxidative stress and l-DOPA reversible motor deficits. Neurobiol. Dis. 74, 66–75. https://doi.org/10.1016/j.nbd.2014.10.016.
Meléndez, C., Muñoz, P., Segura-Aguilar, J., 2019. DT-diaphorase prevents aminochrome-induced lysosome dysfunction in SH-SY5Y cells. Neurotox. Res. 255–259.
Mexas, L.M., Florang, V.R., Doorn, J.A., 2011. Inhibition and covalent modification of tyrosine hydroxylase by 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite. Neurotoxicology 32, 471–477.
Michel, T.M., Käsbauer, L., Gsell, W., Jecel, J., Sheldrick, A.J., Cortese, M., Nickl-Jockschat, T., Grünblatt, E., Riederer, P., 2014. Aldehyde dehydrogenase 2 in sporadic Parkinson's disease. Park. Relat. Disord. 20 (Suppl. 1), S68–S72.
Mor, D.E., Daniels, M.J., Ischiropoulos, H., 2019. The usual suspects, dopamine and alpha-synuclein, conspire to cause neurodegeneration. Mov. Disord. https://doi.org/10.1002/mds.27607. 2019 Jan 11.
Muñoz, P., Cardenas, S., Huenchuguala, S., Briceño, A., Couve, E., Paris, I., Segura-Aguilar, J., 2015. DT-diaphorase prevents aminochrome-induced alpha-synuclein oligomer formation and neurotoxicity. Toxicol. Sci. 145, 37–47.
Muñoz, P., Huenchuguala, S., Paris, I., Segura-Aguilar, J., 2012b. Dopamine oxidation and autophagy. Parkinsons Dis 920953. https://doi.org/10.1155/2012/920953.
Muñoz, P., Paris, I., Sanders, L.H., Greenamyre, J.T., Segura-Aguilar, J., 2012a. Overexpression of VMAT-2 and DT-diaphorase protects substantia nigra-derived cells against aminochrome neurotoxicity. Biochim. Biophys. Acta 1822, 1125–1136.
Muñoz, P.S., Segura-Aguilar, J., 2017. DT-diaphorase protects against autophagy induced by aminochrome-dependent alpha-synuclein oligomers. Neurotox. Res. 32, 362–367.
Nopparat, C., Porter, J.E., Ebadi, M., Govitrapong, P., 2014. 1-Methyl-4-phenylpyridinium-induced cell death via autophagy through a Bcl-2/Beclin 1 complex-dependent pathway. Neurochem. Res. 39, 225–232. https://doi.org/10.1007/s11064-013-1208-8.
Norris, E.H., Giasson, B.I., 2005. Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations. J. Biol. Chem. 280, 21212–22129.
Ochs, S.D., Westfall, T.C., Macarthur, H., 2005. The separation and quantification of aminochromes using high-pressure liquid chromatography with electrochemical detection. J. Neurosci. Methods 142, 201–208.
Olanow, C.W., Bartus, R.T., Baumann, T.L., Factor, S., et al., 2015a. Gene delivery of neurturin to putamen and substantia nigra in Parkinson disease: a double-blind, randomized, controlled trial. Ann. Neurol. 2, 248–257.
Olanow, C.W., Bartus, R.T., Volpicelli-Daley, L.A., Kordower, J.H., 2015b. Trophic factors for Parkinson's disease: to live or let die. Mov. Disord. 30, 1715–1724.
Panneton, W.M., Kumar, V.B., Gan, Q., Burke, W.J., Galvin, J.E., 2010. The neurotoxicity of DOPAL: behavioral and stereological evidence for its role in Parkinson disease pathogenesis. PLoS One 5, e15251.
Paris, I., Muñoz, P., Huenchuguala, S., Couve, E., Sanders, L.H., Greenamyre, J.T., Caviedes, P., Segura-Aguilar, J., 2011. Autophagy protects against aminochrome-induced cell death in substantia nigra-derived cell line. Toxicol. Sci. 121, 376–388.
Paris, I., Perez-Pastene, C., Cardenas, S., Iturriaga-Vasquez, P., Muñoz, P., Couve, E., Caviedes, P., Segura-Aguilar, J., 2010. Aminochrome induces disruption of actin, alpha-, and beta-tubulin cytoskeleton networks in substantia-nigra-derived cell line. Neurotox. Res. 18, 82–92.
Park, A., Stacy, M., 2015. Disease-modifying drugs in Parkinson's disease. Drugs 75, 2065–2071.
Pifl, C., Rajput, A., Reither, H., Blesa, J., Cavada, C., Obeso, J.A., Rajput, A.H., Hornykiewicz, O., 2014. Is Parkinson's disease a vesicular dopamine storage disorder? Evidence from a study in isolated synaptic vesicles of human and nonhuman primate striatum. J. Neurosci. 34, 8210–8218.
Plotegher, N., Berti, G., Ferrari, E., Tessari, I., Zanetti, M., Lunelli, L., et al., 2017. L. DOPAL derived alpha-synuclein oligomers impair synaptic vesicles physiological function. Sci. Rep. 7, 40699.
Rabinovic, A.D., Lewis, D.A., Hastings, T.G., 2000. Role of oxidative changes in the degeneration of dopamine terminals after injection of neuro-toxic levels of dopamine. Neuroscience 101, 67–76.
Rosengren, E., Linder-Eliasson, E., Carlsson, A., 1985. Detection of 5-S-cysteinyldopamine in human brain. J. Neural Transm. 63, 247–253.
Santos, C.C., Araújo, F.M., Ferreira, R.S., Silva, V.,B., Silva, J.,H., Grangeiro, M.S., Soares, É.N., et al., 2017. Aminochrome induces microglia and astrocyte activation. 2017. Toxicol. Vitro S0887-2333 (17) 30089-9.
Segura-Aguilar, J., Baez, S., Widersten, M., Welch, C.J., Mannervik, B., 1997. Human class Mu glutathione transferases, in particular isoenzyme M2-2, catalyze detoxication of the dopamine metabolite aminochrome. J. Biol. Chem. 272, 5727–5731.
Segura-Aguilar, J., Huenchuguala, S., 2018. Aminochrome induces irreversible mitochondrial dysfunction by inducing autophagy dysfunction in Parkinson's disease. Front. Neurosci. 12, 106. https://doi.org/10.3389/fnins.2018.00106.
Segura-Aguilar, J., Lind, C., 1989. On the mechanism of the Mn3(+)-induced neurotoxicity of dopamine:prevention of quinone-derived oxygen toxicity by DT diaphorase and superoxide dismutase. Chem. Biol. Interact. 72, 309–324.
Segura-Aguilar, J., Paris, I., Muñoz, P., Ferrari, E., Zecca, L., Zucca, F.A., 2014. Protective and toxic roles of dopamine in Parkinson's disease. J. Neurochem. 129, 898–915.
Sulzer, D., Bogulavsky, J., Larsen, K.E., Behr, G., Karatekin, E., Kleinman, M.H., Turro, N., Krantz, D., Edwards, R.H., Greene, L.A., Zecca, L., 2000. Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. Proc. Natl. Acad. Sci. U.S.A. 97, 11869–11874.
Touchette, J.C., Breckenridge, J.M., Wilken, G.H., Macarthur, H., 2016. Direct intranigral injection of dopaminochrome causes degeneration of dopamine neurons. Neurosci. Lett. 612, 178–184.
Tse, D.C.S., McCreery, R.L., Adams, R.N., 1976. Potential oxidative pathways of brain catecholamines. J. Med. Chem. 19 37–34.
Van Laar, V.S., Mishizen, A.J., Cascio, M., Hastings, T.G., 2009. Proteomic identification of dopamine-conjugated proteins from isolated rat brain mitochondria and SH-SY5Y cells. Neurobiol. Dis. 34, 487–500.
Wang, Y.M., Pu, P., Le, W.D., 2007. ATP depletion is the major cause of MPP+ induced dopamine neuronal death and worm lethality in alpha-synuclein transgenic C. elegans. Neurosci. Bull. 23, 329–335.
Werner-Allen, J.W., DuMond, J.F., Levine, R.L., Bax, A., 2016. Toxic dopamine metabolite DOPAL forms an unexpected dicatechol pyrrole adduct with lysines of α-synuclein. Angew Chem. Int. Ed. Engl. 55, 7374–7378.
Werner-Allen, J.W., Levine, R.L., Bax, A., 2017. Superoxide is the critical driver of DOPAL autoxidation, lysyl adduct formation, and crosslinking of α-synuclein. Biochem. Biophys. Res. Commun. 487, 281–286.
Werner-Allen, J.W., Monti, S., DuMond, J.F., Levine, R.L., Bax, A., 2018. Isoindole linkages provide a pathway for DOPAL-mediated cross-linking of α-synuclein. Biochemistry 57, 1462–1474.
Whitehead, R.E., Ferrer, J.V., et al., 2001. Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. J. Neurochem. 76,

1242–1251.
Williams, A., 1986. MPTP toxicity: clinical features. J. Neural Transm. Suppl. 20, 5–9.
Xie, Q., Liu, M., Yan, Y.F., Shen, X., Wang, E.S., 2019 Feb. Exogenous tetranectin protects against 1-methyl-4-phenylpyridine-induced neurotoxicity by inhibiting apoptosis and autophagy through ribosomal protein S6 kinase beta-1. World Neurosurg 122, e375–e382.
Xiong, R., Siegel, D., Ross, D., 2014. Quinone-induced protein handling changes: implications for major protein handling systems in quinone-mediated toxicity. Toxicol. Appl. Pharmacol. 280, 285–295.
Zafar, K.S., Siegel, D., Ross, D., 2006. A potential role for cyclized quinones derived from dopamine, DOPA, and 3,4- dihydroxyphenylacetic acid in proteasomal inhibition. Mol. Pharmacol. Pharmacol. 70, 1079–1086.
Zecca, L., Fariello, R., et al., 2002. The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. FEBS Lett. 510, 216–220.
Zhou, Z.D., Lim, T.M., 2009. Dopamine(DA) induced irreversible proteasome inhibition via DA derived quinones. Free Radic. Res. 43, 417–430.
Zhao, M., Chen, J., Mao, K., She, H., Ren, Y., Gui, C., et al., 2019. Mitochondrial calcium dysfunction contributes to autophagic cell death induced by MPP+ via AMPK pathway. Biochem. Biophys. Res. Commun. 509, 390–394.
Zhu, J.H., Horbinski, C., Guo, F., Watkins, S., Uchiyama, Y., Chu, C.T., 2007. Regulation of autophagy by extracellular signal-regulated protein kinases during 1-methyl-4-phenylpyridinium-induced cell death. Am. J. Pathol. 170, 75–86.
Zilocchi, M., Finzi, G., Lualdi, M., Sessa, F., Fasano, M., Alberio, T., 2018. Mitochondrial alterations in Parkinson's disease human samples and cellular models. Neurochem. Int. 118, 61–72.