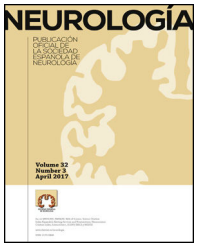
Neurología. 2017;32(8):533—539

SOCIEDAD ESPAÑOLA DE NEUROLOGIA

NEUROLOGÍA

www.elsevier.es/neurologia



REVIEW ARTICLE

The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model☆,☆☆

D. Hernandez-Baltazar[a,b,*], **L.M. Zavala-Flores**[c], **A. Villanueva-Olivo**[d]

[a] *Cátedra CONACyT, Dirección Adjunta de Desarrollo Científico CONACyT, México, D. F., Mexico*
[b] *Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, Mexico*
[c] *Centro de Investigación Biomédica del Noreste, IMSS, Monterrey, Nuevo León, Mexico*
[d] *Departamento de Histología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico*

Received 27 November 2014; accepted 15 June 2015
Available online 23 August 2017

KEYWORDS
Apoptosis;
Nigrostriatal pathway;
Neurodegeneration;
Substantia nigra;
Neuroinflammation;
Oxidative stress

Abstract The neurotoxin 6-hydroxydopamine (6-OHDA) is widely used to induce models of Parkinson's disease (PD). We now know that the model induced by 6-OHDA does not include all PD symptoms, although it does reproduce the main cellular processes involved in PD, such as oxidative stress, neurodegeneration, neuroinflammation, and neuronal death by apoptosis. In this review we analyse the factors affecting the vulnerability of dopaminergic neurons as well as the close relationships between neuroinflammation, neurodegeneration, and apoptosis in the 6-OHDA model. Knowledge of the mechanisms involved in neurodegeneration and cell death in this model is the key to identifying potential therapeutic targets for PD.

PALABRAS CLAVE
Apoptosis;
Vía nigroestriatal;
Neurodegeneración;
Substantia nigra;
Neuroinflamación;
Estrés oxidativo

El modelo de 6-hidroxidopamina y la fisiopatología parkinsoniana: nuevos hallazgos en un viejo modelo

Resumen El neurotóxico 6-hidroxidopamina (6-OHDA) ha sido utilizado para generar modelos de la enfermedad de Parkinson (EP). A la fecha se ha establecido que si bien el modelo neurodegenerativo inducido por la 6-OHDA no reproduce la totalidad de síntomas de la enfermedad, sí replica procesos celulares tales como el estrés oxidativo, la neurodegeneración, la neuroinflamación y la muerte neuronal por apoptosis. En esta revisión se contempla el análisis de los

☆ Please cite this article as: Hernandez-Baltazar D, Zavala-Flores LM, Villanueva-Olivo A. El modelo de 6-hidroxidopamina y la fisiopatología parkinsoniana: nuevos hallazgos en un viejo modelo. Neurología. 2017;32:533—539.
☆☆ This study has not been presented at the SEN's Annual Meeting or at any other conferences or congresses.
* Corresponding author.
E-mail address: dan.hernandez.baltazar@gmail.com (D. Hernandez-Baltazar).

factores que influyen en la vulnerabilidad de las neuronas dopaminérgicas, así como la estrecha relación entre el proceso neurodegenerativo, el neuroinflamatorio y la apoptosis ocasionada por la 6-OHDA. El conocimiento de los mecanismos involucrados en la neurodegeneración y la muerte celular en este modelo es relevante para definir posibles blancos terapéuticos para EP.

Background

Parkinson's disease (PD) is a chronic degenerative disease causing motor and non-motor symptoms.[1] This condition affects multiple nuclei of the central nervous system, including the dorsal motor nucleus of the vagus, raphe nuclei, locus coeruleus, pedunculopontine nucleus, retrorubral nucleus, parabrachial nucleus, ventral tegmental area (VTA), and the substantia nigra pars compacta (SNpc).[2] PD may be caused by a wide range of factors. Undeniable signs of PD include progressive degeneration of dopaminergic neurons in the nigrostriatal pathway, presence of Lewy bodies,[3] and generalised damage to the neuronal circuits that control movement.[1]

Animal models reproducing the main cellular processes of PD, such as oxidative stress, neurodegeneration, neuroinflammation, and cell death, have been used to assess neurodegeneration in PD. Some of the most widely used neurotoxic compounds are: 1) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is converted into 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B (MAO-B)[4,5]; and 2) 6-hydroxydopamine (6-OHDA).[6,7] MPTP crosses the blood-brain barrier (BBB),[8] causing not only damage to the nigrostriatal pathway, but also loss of striatal GABAergic neurons[9] and neurons in the VTA and retrorubral nucleus, as well as reactive gliosis.[10,11] 6-OHDA is the drug most frequently used to induce neurodegeneration of the nigrostriatal system, due to its inability to cross the BBB and its selectivity for dopaminergic neurons of the SNpc.

6-OHDA is a highly oxidisable dopamine analogue that may be uptaken by the dopamine transporter, which allows selective damage to catecholaminergic neurons, such as dopaminergic neurons of the SNpc.[12] To date, 3 mechanisms have been proposed to explain the cytotoxic effect of 6-OHDA: 1) intra- or extracellular auto-oxidation of 6-OHDA, which favours the production of hydrogen peroxide and superoxide and hydroxyl radicals[8]; 2) formation of hydrogen peroxide due to the action of monoamine oxidase,[13] and 3) direct inhibition of mitochondrial respiratory chain complex I.[14] These mechanisms may act independently or in combination to generate reactive oxygen species (ROS).[15] The resulting oxidative stress (OS) may be intensified by an increase in cytoplasmic free calcium (as a result of either glutamate excitotoxicity or loss of mitochondrial membrane permeability), finally inducing cell death.[16]

Ungerstedt[6] showed in 1968 that intracerebral stereotactic injection with 6-OHDA causes degeneration of the nigrostriatal pathway. To assess the degenerative effects and mechanisms of 6-OHDA in vivo, 3 lesion models have been developed: 1) lesions to the medial forebrain bundle[17,18]; 2) lesions to the substantia nigra[19,20], and 3) intrastriatal lesions.[21–24] Lesions to the medial forebrain bundle and substantia nigra, though useful for demonstrating the immediate effects of 6-OHDA, provoke rapid, generalised degeneration of the damaged nucleus.[7] These lesion models are therefore not suitable for studying the mechanisms underlying neurotoxicity (and death) due to OS in the long term (21 days after the lesion). However, unilateral[25–28] or bilateral[29,30] intrastriatal injections with 6-OHDA do cause progressive loss of dopaminergic neurons of the SNpc, simulating the nigrostriatal damage observed in PD.

By convention, the rat striatum has been subdivided in 2 regions, the dorsomedial and the ventrolateral regions, which are innervated by specific nuclei. The ventrolateral region receives afferences from the motor and sensiromotor areas of the cortex and is innervated exclusively by neurons originating from the SNpc.[23,31] The dorsomedial region of the striatum is innervated by neurons originating from the SNpc, VTA, frontal cortical area, and limbic system.[23] This explains why 6-OHDA lesions to the dorsomedial striatum have a general effect on locomotion and drug-induced spinning behaviour (amphetamine and apomorphine), whereas lesions to the ventrolateral striatum have marked effects on movement onset, sensorimotor orientation, and fine motor behaviour, all of which are typical signs of PD.[12,23]

In 1998, Kirik et al.[23] established the optimal parameters for inducing unilateral lesions to the ventrolateral striatum with a single dose of 20 μg, or 6 μg doses of 6-OHDA injected at 3 different sites in the striatum. These researchers concluded that the effect of intrastriatal injections depends on the dose and site of the lesion. They observed that a dose administered at a single site of the striatum causes an 80% decrease in striatal innervation and an almost 90% loss of nigral dopaminergic neurons, whereas a dose administered at several sites causes damages to extrastriatal nuclei such as the globus pallidus.[23]

Although intrastriatal lesions mainly affect dopaminergic neurons of the SNpc, they also lead to a decrease in VTA dopaminergic neurons, which constitute the mesolimbic pathway and innervate the nucleus accumbens.[32,33] Loss of VTA dopaminergic neurons does not exceed 20% and damage does not increase with time, as has been observed in the SNpc. Therefore, the intrastriatal lesion model is the

most frequently used model for demonstrating neurodegeneration and cell death mechanisms over a long period of time; we should not forget, however, that the percentage of dopaminergic neuronal loss in the SNpc and collateral damages in the VTA will depend on the site of the lesion and the dose administered.[23]

In view of the fact that the 6-OHDA model does not reproduce presence of Lewy bodies,[34] several alpha-synuclein mouse models have been established, including knockdown models,[35] gene expression models,[36] and models involving intracerebral injections with alpha-synuclein.[37] Given that this protein is a crucial component of Lewy bodies, these models may be key to understanding nigrostriatal pathway degeneration and its impact on other brain nuclei; further research is needed on this topic.

The 6-OHDA model has been used to demonstrate the proof of principle of neurotrophic factor therapy (NFT). This treatment consists of the targeted delivery of genes coding for neurotrophic factors (for example the brain-derived neurotrophic factor [BDNF],[38] the glial cell-derived neurotrophic factor [GDNF],[39–42] and the cerebral dopamine neurotrophic factor [CDNF][43,44]) through nanoparticles[45,46] or viral and non-viral vectors.[2,3] The aim of NFT in the 6-OHDA model is to halt neurodegeneration and stimulate functional regeneration of the nigrostriatal system.[47,48] We should therefore continue identifying the mechanisms of 6-OHDA-induced OS, neurodegeneration, and neuronal death; knowledge on this topic is key to determine the molecular mechanisms underlying the new therapies for PD.

Development

Vulnerability of dopaminergic neurons to 6-OHDA

Dopaminergic neurons in the SNpc are vulnerable to 6-OHDA-induced OS, since they present high baseline ROS levels and low levels of glutathione peroxidase, an enzyme that converts hydrogen peroxide to water to prevent ROS damage.[49] Dopamine, in turn, is highly susceptible to autooxidation and conversion to neuromelanin, which promotes the formation of hydroxyl radicals (OH−). When neuromelanin combines with iron, which normally accumulates at high concentrations in dopaminergic neurons,[50,51] it affects their ability to remove OH. However, not all dopaminergic neurons in the SNpc are vulnerable to 6-OHDA toxicity; the SNpc contains subpopulations of dopaminergic neurons that express such calcium-binding proteins as calretinin (CR) and calbindin (CB), both of which reduce the accumulation of intracellular calcium, preventing glutamate excitotoxicity and ultimately inhibiting the cytotoxic effects of 6-OHDA.[52,53] The precise number of neurons constituting these dopaminergic subpopulations within the SNpc is as yet unknown; presence of these subpopulations has been suggested to explain the presence of 10%[23] dopaminergic neurons in the SNpc after treatment with 6-OHDA, regardless of the dose and site of injection of the neurotoxic compound.[22]

According to several in vivo studies, OS mediates the cytotoxic effects of 6-OHDA. Microdialysis and HPLC studies of the caudate nucleus of adult rats show that perfusion of 100 μM 6-OHDA through a microdialysis probe for 60 minutes increases production of free radicals. Under these conditions, the DNA of SNpc dopaminergic neurons is also damaged.[54] Recent studies have shown that intrastriatal injection with 6 μg 6-OHDA in rats causes a time-dependent increase in the indices of protein oxidation and lipid peroxidation.[55] It has therefore been suggested that these biochemical processes originated by mitochondrial OS lead to both neuroinflammation and cell death by apoptosis.[1]

Neuroinflammation

Neuroinflammation has been evaluated using such glial cell markers as GFAP for astrocytes[56,57] and OX-42 for microglia.[58,59] 6-OHDA-induced activation of these glial populations has been found to occur from the third day after the lesion[22] up to the third week postlesion.[57] Neuroinflammation has been shown to precede death of nigral dopaminergic neurons (2 weeks after the lesion),[57] probably to minimise cell damage. Other studies suggest that increased activation of glial cells and subsequent release of proinflammatory and anti-inflammatory cytokines at the site of the lesion may increase 6-OHDA cytotoxicity.[60] Studying neuroinflammation in 6-OHDA models is essential to determine alternative therapeutic targets in PD.

Apoptosis in the SNpc

Tyrosine hydroxylase (TH), the rate-limiting enzyme of dopamine synthesis, is a marker for dopaminergic phenotypes.[23] The hypothesis that 6-OHDA injection causes death of dopaminergic neurons in the SNpc is based on the decrease in the number of TH+ neurons.[19,61] Most researchers using 6-OHDA in vivo assert that this neurotoxic compound causes cell death[22,62–65]; a smaller number of researchers, in contrast, have detected no markers of cell death,[23,66] which may suggest loss of phenotype. However, this depends on the dose and the lesion model used.

Cell death is the final result of 6-OHDA-induced cytotoxicity. Some studies try to demonstrate activation of apoptosis in the SNpc using silver staining or terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining after intrastriatal administration of 6-OHDA.[64,67,68] However, these studies do not use other markers of apoptosis in addition to TUNEL staining to support the hypothesis that 6-OHDA induces apoptosis. Furthermore, TUNEL staining also identifies necrotic cells; therefore, we cannot conclude that 6-OHDA causes apoptosis based on the results of this technique, especially in the light of in vitro studies showing that the dose of 6-OHDA frequently used in vivo causes necrosis.[69,70] According to a recent study with rats, in addition to TH loss, 6-OHDA injections to the striatum cause also loss of cytoskeleton integrity in dopaminergic neurons of the SNpc,[22] which demonstrates cell death by apoptosis. Although many authors agree that apoptosis is the main type of cell death in 6-OHDA models, necrosis and autophagy[71–73] have also been analysed in the context of in vivo 6-OHDA cell death. Given the variety of experimental models, it is not possible to determine the percentage of dopaminergic neurons in the SNpc affected by one or another type of death. However, convergence of several types of death may explain

activation of the neuroinflammatory process, a topic that merits further study.

Apoptosis: caspase-3 and glycogen synthase kinase 3β

Apoptosis is a process of programmed cell death triggered by 2 signalling pathways: the intrinsic and the extrinsic pathways.[74] The intrinsic pathway involves mitochondrial damage and subsequent cytochrome C release, which promotes the activation of proapoptotic proteins. The extrinsic pathway requires the activation of death receptors in the cell membrane and the subsequent activation of a complex signalling cascade.[11,74] Though independent, these 2 pathways converge in the activation of effector caspases-3, -6, or -7.[75,76]

Caspase-3 is the main effector caspase in neurons and its activation has been demonstrated by the in vitro and in vivo administration of neurotoxic compounds.[75,77,78] In vivo studies have detected caspase-3 activation one week after instrastriatal administration of 6-OHDA to rats.[55,57] Most in vivo studies demonstrate caspase-3 expression in different models of cell death, suggesting that caspase- 3 activation is involved in programmed cell death in neurons of the SNpc.[78–80] However, recent studies have failed to confirm activation of caspase-3 or caspase-9, concluding that these caspases are not involved in apoptosis of dopaminergic neurons in the SNpc.[81,82] There is even greater controversy in the light of recent evidence that caspase-3 is involved in non-apoptotic functions, such as microglial activation.[83,84] Although most authors agree that caspase-3 participates in 6-OHDA-induced neurodegeneration, the question remains whether its expression leads to neuronal death only. Other markers of apoptosis are therefore necessary; in this context, we should mention the study of glycogen synthase kinase 3β (GSK-3β).

GSK-3β is involved in the signalling pathway of neuronal apoptosis triggered by OS,[85] a pivotal factor in the pathogenesis of PD.[86] GSK-3β is activated by phosphorylation of tyrosine 216 residue (Y216), located in the kinase domain, and inactivated by phosphorylation of serine 9 (S9).[85] A recent study showed that caspase-3 and GSK-3β are involved in the apoptotic process induced by 6-OHDA in vivo.[22] A single 20 μg dose administered to a rat's striatum was observed to cause loss of cytoskeleton integrity, decreased TH immunoreactivity, and a progressive decrease in immunoreactivity to NeuN (a marker of neuronal lineage) in dopaminergic neurons in the SNpc.[22]

Furthermore, Kim et al.[82] observed atrophy and progressive death of dopaminergic neurons which was dependent on nuclear translocation of apoptosis-inducing factor (AIF), but found no signs of apoptosis in the form of caspase-3 activation or cytoplasmic release of cytochrome C. These researchers also showed that death induced by 6-OHDA in dopaminergic neurons is mediated by Bax-dependent AIF activation.[82] In their study, AIF activation suggested the involvement of regulated necrosis. The controversy on whether death is dependent on or independent from caspase-3 may be explained by the different doses, experimental models, and lesion sites used in the literature.

Although most evidence suggests that caspase-3 is involved in 6-OHDA-induced apoptosis, other studies suggest that 6-OHDA may also cause neuronal death by apoptosis (independent from caspase-3) or other cell death processes (necrosis and autophagy) in vivo.

Conclusion

The 6-OHDA model reproduces several cell processes identified in PD and therefore constitutes a key model for exploring the molecular basis of cytotoxicity and studying the cell processes activated by OS (neuroinflammation and neuronal death). In conclusion, this model is useful for understanding the mechanisms underlying novel therapies for PD.

Funding

This study was financed by the Consejo Nacional de Ciencia y Tecnología (CONACyT) and the Instituto de Neuroetología of Universidad Veracruzana in Mexico (''Cátedra CONACyT'' grant to project no. 1840 by Daniel Hernández-Baltazar).

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Dexter DT, Jenner P. Parkinson disease: from pathology to molecular disease mechanisms. Free Radic Res Med. 2013;62:132–44.
2. Sulzer D, Surmeier DJ. Neuronal vulnerability, pathogenesis, and Parkinson's disease. Mov Disord. 2013;28:41–50.
3. Double KL. Neuronal vulnerability in Parkinson's disease. Parkinsonism Relat Disord. 2012;18 Suppl. 1:S52–4.
4. Chiueh CC, Miyake H, Peng MT. Role of dopamine autoxidation, hydroxyl radical generation, and calcium overload in underlying mechanisms involved in MPTP-induced parkinsonism. Adv Neurol. 1993;60:251–8.
5. Langston JW, Ballard PA Jr. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. N Engl J Med. 1983;309:310.
6. Ungerstedt U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol. 1968;5:107–10.
7. Blesa J, Phani S, Jackson-Lewis V, Przedborski S. Classic and new animal models of Parkinson's disease. J Biomed Biotechnol. 2012;2012:845618.
8. Blum D, Torch S, Lambeng N, Nissou M, Benabid AL, Sadoul R, et al. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog Neurobiol. 2001;65:135–72.
9. Altar CA, Heikkila RE, Manzino L, Marien MR. 1-Methyl-4-phenylpyridine (MPP+): regional dopamine neuron uptake, toxicity, and novel rotational behavior following dopamine receptor proliferation. Eur J Pharmacol. 1986;131:199–209.
10. Ter Horst GJ, Knigge MF, van der Wal A. Neurochemical lesioning in the rat brain with iontophoretic injection of the 1-methyl-4-phenylpyridinium ion (MPP+). Neurosci Lett. 1992;141:203–7.

11. Blesa J, Przedborski S. Parkinson's disease: animal models and dopaminergic cell vulnerability. Front Neuroanat. 2014;8:155.
12. Blandini F, Armentero MT. Animal models of Parkinson's disease. FEBS J. 2012;279:1156—66.
13. Chiba K, Trevor A, Castagnoli N Jr. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem Biophys Res Commun. 1984;120:574—8.
14. Jenner P, Schapira AH, Marsden CD. New insights into the cause of Parkinson's disease. Neurology. 1992;42:2241—50.
15. Harrison JF, Hollensworth SB, Spitz DR, Copeland WC, Wilson GL, LeDoux SP. Oxidative stress-induced apoptosis in neurons correlates with mitochondrial DNA base excision repair pathway imbalance. Nucleic Acids Res. 2005;33:4660—71.
16. Singh S, Kumar S, Dikshit M. Involvement of the mitochondrial apoptotic pathway and nitric oxide synthase in dopaminergic neuronal death induced by 6-hydroxydopamine and lipopolysaccharide. Redox Rep. 2010;15:115—22.
17. Perese DA, Ulman J, Viola J, Ewing SE, Bankiewicz KS. A 6-hydroxydopamine-induced selective parkinsonian rat model. Brain Res. 1989;494:285—93.
18. Venero JL, Revuelta M, Cano J, Machado A. Time course changes in the dopaminergic nigrostriatal system following transection of the medial forebrain bundle: detection of oxidatively modified proteins in substantia nigra. J Neurochem. 1997;68:2458—68.
19. Stanic D, Finkelstein DI, Bourke DW, Drago J, Horne MK. Timecourse of striatal re-innervation following lesions of dopaminergic SNpc neurons of the rat. Eur J Neurosci. 2003;18:1175—88.
20. Stanic D, Parish CL, Zhu WM, Krstew EV, Lawrence AJ, Drago J, et al. Changes in function and ultrastructure of striatal dopaminergic terminals that regenerate following partial lesions of the SNpc. J Neurochem. 2003;86:329—43.
21. Decressac M, Mattsson B, Bjorklund A. Comparison of the behavioural and histological characteristics of the 6-OHDA and alpha-synuclein rat models of Parkinson's disease. Exp Neurol. 2012;235:306—15.
22. Hernandez-Baltazar D, Mendoza-Garrido ME, Martinez-Fong D. Activation of GSK-3beta and caspase-3 occurs in nigral dopamine neurons during the development of apoptosis activated by a striatal injection of 6-hydroxydopamine. PLOS ONE. 2013;8:e70951.
23. Kirik D, Rosenblad C, Bjorklund A. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. Exp Neurol. 1998;152:259—77.
24. Mercanti G, Bazzu G, Giusti P. A 6-hydroxydopamine in vivo model of Parkinson's disease. Methods Mol Biol. 2012;846:355—64.
25. Da Rocha JT, Pinton S, Gai BM, Nogueira CW. Diphenyl diselenide reduces mechanical and thermal nociceptive behavioral responses after unilateral intrastriatal administration of 6-hydroxydopamine in rats. Biol Trace Elem Res. 2013;154:372—8.
26. Healy-Stoffel M, Ahmad SO, Stanford JA, Levant B. A novel use of combined tyrosine hydroxylase and silver nucleolar staining to determine the effects of a unilateral intrastriatal 6-hydroxydopamine lesion in the substantia nigra: a stereological study. J Neurosci Methods. 2012;210:187—94.
27. Kelsey JE, Langelier NA, Oriel BS, Reedy C. The effects of systemic, intrastriatal, and intrapallidal injections of caffeine and systemic injections of A2A and A1 antagonists on forepaw stepping in the unilateral 6-OHDA-lesioned rat. Psychopharmacology (Berl). 2009;201:529—39.
28. Heuer A, Smith GA, Lelos MJ, Lane EL, Dunnett SB. Unilateral nigrostriatal 6-hydroxydopamine lesions in mice. I: Motor impairments identify extent of dopamine depletion at three different lesion sites. Behav Brain Res. 2012;228:30—43.
29. Roedter A, Winkler C, Samii M, Walter GF, Brandis A, Nikkhah G. Comparison of unilateral and bilateral intrastriatal 6-hydroxydopamine-induced axon terminal lesions: evidence for interhemispheric functional coupling of the two nigrostriatal pathways. J Comp Neurol. 2001;432:217—29.
30. Amalric M, Moukhles H, Nieoullon A, Daszuta A. Complex deficits on reaction time performance following bilateral intrastriatal 6-OHDA infusion in the rat. Eur J Neurosci. 1995;7:972—80.
31. Deumens R, Blokland A, Prickaerts J. Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol. 2002;175:303—17.
32. Brichta L, Greengard P. Molecular determinants of selective dopaminergic vulnerability in Parkinson's disease: an update. Front Neuroanat. 2014;8:152.
33. Walsh JJ, Han MH. The heterogeneity of ventral tegmental area neurons: projection functions in a mood-related context. Neuroscience. 2014;282C:101—8.
34. Lindgren HS, Lelos MJ, Dunnett SB. Do alpha-synuclein vector injections provide a better model of Parkinson's disease than the classic 6-hydroxydopamine model. Exp Neurol. 2012;237:36—42.
35. Tai Y, Chen L, Huang E, Liu C, Yang X, Qiu P, et al. Protective effect of alpha-synuclein knockdown on methamphetamine-induced neurotoxicity in dopaminergic neurons. Neural Regen Res. 2014;9:951—8.
36. He Q, Koprich JB, Wang Y, Yu WB, Xiao BG, Brotchie JM, et al. Treatment with trehalose prevents behavioral and neurochemical deficits produced in an AAV alpha-synuclein rat model of Parkinson's disease. Mol Neurobiol. 2016;53:2258—68.
37. Roostaee A, Beaudoin S, Staskevicius A, Roucou X. Aggregation and neurotoxicity of recombinant alpha-synuclein aggregates initiated by dimerization. Mol Neurodegener. 2013;8:5.
38. Razgado-Hernandez LF, Espadas-Alvarez AJ, Reyna-Velazquez P, Sierra-Sanchez A, Anaya-Martinez V, Jimenez-Estrada I, et al. The transfection of BDNF to dopamine neurons potentiates the effect of dopamine d3 receptor agonist recovering the striatal innervation, dendritic spines and motor behavior in an aged rat model of Parkinson's disease. PLOS ONE. 2015;10:e0117391.
39. Chen SS, Yang C, Hao F, Li C, Lu T, Zhao LR, et al. Intrastriatal GDNF gene transfer by inducible lentivirus vectors protects dopaminergic neurons in a rat model of parkinsonism. Exp Neurol. 2014;261:87—96.
40. Herran E, Requejo C, Ruiz-Ortega JA, Aristieta A, Igartua M, Bengoetxea H, et al. Increased antiparkinson efficacy of the combined administration of VEGF- and GDNF-loaded nanospheres in a partial lesion model of Parkinson's disease. Int J Nanomed. 2014;9:2677—87.
41. Deng X, Liang Y, Lu H, Yang Z, Liu R, Wang J, et al. Co-transplantation of GDNF-overexpressing neural stem cells and fetal dopaminergic neurons mitigates motor symptoms in a rat model of Parkinson's disease. PLOS ONE. 2013;8:e80880.
42. Gonzalez-Barrios JA, Lindahl M, Bannon MJ, Anaya-Martínez V, Flores G, Navarro-Quiroga I, et al. Neurotensin polyplex as an efficient carrier for delivering the human GDNF gene into nigral dopamine neurons of hemiparkinsonian rats. Mol Ther. 2006;14:857—65.
43. Nadella R, Voutilainen MH, Saarma M, Gonzalez-Barrios JA, Leon-Chavez BA, Jiménez JM, et al. Transient transfection of human CDNF gene reduces the 6-hydroxydopamine-induced neuroinflammation in the rat substantia nigra. J Neuroinflamm. 2014;11:209.
44. Cordero-Llana O, Houghton BC, Rinaldi F, Taylor H, Yáñez-Muñoz RJ, Uney JB, et al. Enhanced efficacy of the CDNF/MANF family by combined intranigral overexpression in the 6-OHDA rat model of Parkinson's disease. Mol Ther. 2015;23:244—54.
45. Kulkarni AD, Vanjari YH, Sancheti KH, Belgamwar VS, Surana SJ, Pardeshi CV. Nanotechnology-mediated nose to brain drug

delivery for Parkinson's disease: a mini review. J Drug Target. 2015:1—14.
46. Iqbal A, Ahmad I, Khalid MH, Nawaz MS, Gan SH, Kamal MA. Nanoneurotoxicity to nanoneuroprotection using biological and computational approaches. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2013;31:256—84.
47. Pardo J, Morel GR, Astiz M, Schwerdt JI, León ML, Rodríguez SS, et al. Gene therapy and cell reprogramming for the aging brain: achievements and promise. Curr Gene Ther. 2014;14:24—34.
48. Allen PJ, Feigin A. Gene-based therapies in Parkinson's disease. Neurotherapeutics. 2014;11:60—7.
49. Hirsch EC, Faucheux B, Damier P, Mouatt-Prigent A, Agid Y. Neuronal vulnerability in Parkinson's disease. J Neural Transm. 1997;50:79—88.
50. Double KL, Gerlach M, Schunemann V, Trautwein AX, Zecca L, Gallorini M, et al. Iron-binding characteristics of neuromelanin of the human substantia nigra. Biochem Pharmacol. 2003;66:489—94.
51. Gerlach M, Riederer P, Double KL. Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord. 2008;14 Suppl. 2:S185—8.
52. Nemoto C, Hida T, Arai R. Calretinin and calbindin-D28k in dopaminergic neurons of the rat midbrain: a triple-labeling immunohistochemical study. Brain Res. 1999;846:129—36.
53. Tsuboi K, Kimber TA, Shults CW. Calretinin-containing axons and neurons are resistant to an intrastriatal 6-hydroxydopamine lesion. Brain Res. 2000;866:55—64.
54. Ferger B, Rose S, Jenner A, Halliwell B, Jenner P. 6-hydroxydopamine increases hydroxyl free radical production and DNA damage in rat striatum. Neuroreport. 2001;12:1155—9.
55. Sanchez-Iglesias S, Rey P, Mendez-Alvarez E, Labandeira-Garcia JL, Soto-Otero R. Time-course of brain oxidative damage caused by intrastriatal administration of 6-hydroxydopamine in a rat model of Parkinson's disease. Neurochem Res. 2007;32:99—105.
56. Middeldorp J, Hol EM. GFAP in health and disease. Prog Neurobiol. 2011;93:421—43.
57. Walsh S, Finn DP, Dowd E. Time-course of nigrostriatal neurodegeneration and neuroinflammation in the 6-hydroxydopamine-induced axonal and terminal lesion models of Parkinson's disease in the rat. Neuroscience. 2011;175:251—61.
58. Aguzzi A, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else. Science. 2013;339:156—61.
59. Rodrigues RW, Gomide VC, Chadi G. Astroglial and microglial activation in the wistar rat ventral tegmental area after a single striatal injection of 6-hydroxydopamine. Int J Neurosci. 2004;114:197—216.
60. Stott SR, Barker RA. Time course of dopamine neuron loss and glial response in the 6-OHDA striatal mouse model of Parkinson's disease. Eur J Neurosci. 2014;39:1042—56.
61. Sauer H, Oertel WH. Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience. 1994;59:401—15.
62. Tobon-Velasco JC, Limon-Pacheco JH, Orozco-Ibarra M, Macías-Silva M, Vázquez-Victorio G, Cuevas E, et al. 6-OHDA-induced apoptosis and mitochondrial dysfunction are mediated by early modulation of intracellular signals and interaction of Nrf2 and NF-kappaB factors. Toxicology. 2013;304:109—19.
63. Bernstein AI, Garrison SP, Zambetti GP, O'Malley KL. 6-OHDA generated ROS induces DNA damage and p53- and PUMA-dependent cell death. Mol Neurodegener. 2011;6:2.
64. Marti MJ, Saura J, Burke RE, Jackson-Lewis V, Jiménez A, Bonastre M, et al. Striatal 6-hydroxydopamine induces apoptosis of nigral neurons in the adult rat. Brain Res. 2002;958:185—91.
65. Ichitani Y, Okamura H, Nakahara D, Nagatsu I, Ibata Y. Biochemical and immunocytochemical changes induced by intrastriatal 6-hydroxydopamine injection in the rat nigrostriatal dopamine neuron system: evidence for cell death in the substantia nigra. Exp Neurol. 1994;130:269—78.
66. Kostrzewa RM. Review of apoptosis vs necrosis of substantia nigra pars compacta in Parkinson's disease. Neurotox Res. 2000;2:239—50.
67. Blandini F, Levandis G, Bazzini E, Nappi G, Armentero MT. Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastriatal injection of 6-hydroxydopamine in the rat: new clues from an old model. Eur J Neurosci. 2007;25:397—405.
68. Zuch CL, Nordstroem VK, Briedrick LA, Hoernig GR, Granholm AC, Bickford PC. Time course of degenerative alterations in nigral dopaminergic neurons following a 6-hydroxydopamine lesion. J Comp Neurol. 2000;427:440—54.
69. Charriaut-Marlangue C, Ben-Ari Y. A cautionary note on the use of the TUNEL stain to determine apoptosis. Neuroreport. 1995;7:61—4.
70. Ito Y, Shibata MA, Kusakabe K, Otsuki Y. Method of specific detection of apoptosis using formamide-induced DNA denaturation assay. J Histochem Cytochem. 2006;54: 683—92.
71. In S, Hong CW, Choi B, Jang BG, Kim MJ. Inhibition of mitochondrial clearance and Cu/Zn-SOD activity enhance 6-hydroxydopamine-induced neuronal apoptosis. Mol Neurobiol. 2016;53:777—9.
72. Marin C, Aguilar E. In vivo 6-OHDA-induced neurodegeneration and nigral autophagic markers expression. Neurochem Int. 2011;58:521—6.
73. Giordano S, Darley-Usmar V, Zhang J. Autophagy as an essential cellular antioxidant pathway in neurodegenerative disease. Redox Biol. 2014;2:82—90.
74. Jellinger KA. Cell death mechanisms in neurodegeneration. J Cell Mol Med. 2001;5:1—17.
75. D'Amelio M, Sheng M, Cecconi F. Caspase-3 in the central nervous system: beyond apoptosis. Trends Neurosci. 2012;35:700—9.
76. Han BS, Hong HS, Choi WS, Markelonis GJ, Oh TH, Oh YJ. Caspase-dependent and -independent cell death pathways in primary cultures of mesencephalic dopaminergic neurons after neurotoxin treatment. J Neurosci. 2003;23:5069—78.
77. Cutillas B, Espejo M, Gil J, Ferrer I, Ambrosio S. Caspase inhibition protects nigral neurons against 6-OHDA-induced retrograde degeneration. Neuroreport. 1999;10:2605—8.
78. Oo TF, Siman R, Burke RE. Distinct nuclear and cytoplasmic localization of caspase cleavage products in two models of induced apoptotic death in dopamine neurons of the substantia nigra. Exp Neurol. 2002;175:1—9.
79. Hanrott K, Gudmunsen L, O'Neill MJ, Wonnacott S. 6-Hydroxydopamine-induced apoptosis is mediated via extracellular auto-oxidation and caspase 3-dependent activation of protein kinase Cdelta. J Biol Chem. 2006;281: 5373—82.
80. Jeon BS, Kholodilov NG, Oo TF, Kim SY, Tomaselli KJ, Srinivasan A, et al. Activation of caspase-3 in developmental models of programmed cell death in neurons of the substantia nigra. J Neurochem. 1999;73:322—33.
81. Ebert AD, Hann HJ, Bohn MC. Progressive degeneration of dopamine neurons in 6-hydroxydopamine rat model of Parkinson's disease does not involve activation of caspase-9 and caspase-3. Neurosci Res. 2008;86:317—25.
82. Kim TW, Moon Y, Kim K, Lee JE, Koh HC, Rhyu IJ, et al. Dissociation of progressive dopaminergic neuronal death and behavioral impairments by Bax deletion in a mouse model of Parkinson's diseases. PLOS ONE. 2011;6:e25346.
83. Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, et al. Caspase signalling controls microglia activation and neurotoxicity. Nature. 2011;472:319—24.

84. Venero JL, Burguillos MA, Joseph B. Caspases playing in the field of neuroinflammation: old and new players. Dev Neurosci. 2013;35:88—101.
85. Gomez-Sintes R, Hernandez F, Lucas JJ, Avila J. GSK-3 mouse models to study neuronal apoptosis and neurodegeneration. Front Mol Neurosci. 2011;4:45.
86. Golpich M, Amini E, Hemmati F, Ibrahim NM, Rahmani B, Mohamed Z, et al. Glycogen synthase kinase-3 beta (GSK-3beta) signaling: implications for Parkinson's disease. Pharmacol Res. 2015;97:16—26.