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Review Article

Use of invertebrates to model chemically induced parkinsonism-symptoms

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The prevalence of neurological diseases is currently growing due to the combination of several factor, including poor lifestyle and environmental imbalance which enhance the contribution of genetic factors. Parkinson's disease (PD), a chronic and progressive neurological condition, is one of the most prevalent neurodegenerative human diseases. Development of models may help to understand its pathophysiology. This review focuses on studies using invertebrate models to investigate certain chemicals that generate parkinsonian-like symptoms models. Additionally, we report some preliminary results of our own research on a crustacean (the crab *Ucides cordatus*) and a solitary ascidian (*Styela plicata*), used after induction of parkinsonism with 6-hydroxydopamine and the pesticide rotenone, respectively. We also discuss the advantages, limits, and drawbacks of using invertebrate models to study PD. We suggest prospects and directions for future investigations of PD, based on invertebrate models.

Introduction

Parkinson's disease (PD) is a chronic, progressive, and neurodegenerative condition that has been increasing faster than any other neurological disorder in age-standardized rates of prevalence, disability, and deaths [1–4]. Approximately 8.5 million individuals have PD worldwide, with an estimated 81% increase in the number of individuals affected by PD since 2000 [5]. Additionally, the global prevalence of PD increased ∼155% from 1990 to 2019 [6].

While the etiology of PD is still largely unknown, the condition is multifactorial, with genetic and sporadic (idiopathic) cases contributing to the disease genesis [7]. Genetic cases follow Mendelian inheritance in an autosomal dominant or recessive manner; ∼90% of cases are sporadic, in which the exact pathogenic mechanisms are not completely understood [8]. Although only 10% of PD cases involve known genetic mutations, Giambò et al. [9] proposed that environmental factors largely contribute to the prevalence of this disease, and in fact found that the association between pesticides and exposure to high levels of heavy metals increased the risk of development of sporadic PD [9]. The proposed mechanism was the modulation of the expression levels of different PD (*PARK*) genes and DNA methylation. Certain environmental factors associated with a rural lifestyle (drinking well water and exposure to pesticides) have been identified as possible risk factors for PD [10].

PD pathogenesis consists of a progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*, involving misfolding and abnormal aggregation of α-synuclein, mitochondrial abnormalities in the neurons, neuroinflammation, and oxidative stress [11–14]. Environmental pesticides, such as paraquat and rotenone, and other chemicals, such as 6-hydroxydopamine (6-OHDA) and

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), have been used to generate animal models to help reveal PD pathogenesis [15–17]. However, although the use of these models has generated new knowledge in this field, an effective treatment or cure for the disease has yet to be found [18,19].

Because of ethical issues regarding the use of vertebrate animals, invertebrates have emerged as models. Although less frequently used to study PD, invertebrates offer several advantages, such as short lifespans, ease of maintenance and manipulation, low cost, and genetic homology to vertebrates [20,21]. Additionally, this group of animals concords with the principles of human experimental techniques based on the 3 R's (replacement, reduction, and refinement) coined by Russel and Burch [22], promoting the use of animals for human treatment purposes. Therefore, chemicals used in mammal models have been applied in invertebrates to study cause-effect, disclose pathogenic mechanisms, screen drug treatments, reveal the function of genes, and induce parkinsonian signs [23–25].

In this review, we discuss recent advancements in the use of invertebrates as models to study parkinsonism after the disease was induced with certain chemicals, particularly 6-OHDA, MPTP, rotenone, and paraquat. Investigation of the effects of pesticides on invertebrates provide valuable information, not only because these animals are useful models, but also because they respond to (and therefore may be used as bioindicators) and are impacted by environmental toxic agents. We focus on some of the invertebrate models that have been studied: flatworms and nematodes, mollusks, arthropods, and solitary ascidians, and describe some of our preliminary results using the crab *Ucides cordatus* and the ascidian *Styela plicata*. We review some data published in the articles that illustrate how invertebrate models may help to understand the pathophysiology of PD. We also discuss the implications of these studies for humans, stressing the advantages of using them with a special focus on neuroprotection.

Flatworms

Planarians have an almost unlimited capacity to regenerate tissues. This phenomenon has long fascinated naturalists; in the Western Hemisphere alone, their documented history spans more than 200 years. Pieces of planarians can regenerate into a complete organism, including the cephalic ganglion, their 'brain', as reviewed by Elliott and Alvarado [26]. Some decades ago, the damage to dopaminergic cells in planarians following exposure to 6-OHDA [27], MPTP, or rotenone [28] was evidenced. These studies indirectly revealed that dopaminergic cells and dopaminergic receptors occur in planarians. In 2007, Nishimura et al. [29] identified genes that code their dopamine biosynthetic enzymes. Additionally, neurochemical and histochemical data indicated the presence of several neurotransmitter-receptor systems, showing that planarians may contribute significantly to knowledge of neuropharmacology, including the dopaminergic system [30].

In 2011, Nishimura et al. [31] examined cellular aspects of dopaminergic neurogenesis/regeneration from stem cells after selective degeneration of dopaminergic neurons by 6-OHDA. These observations suggest that planarians are suitable animal models for analyzing the system that recognizes the ablation of dopaminergic signals and the system for recruitment of new dopaminergic neurons. Interestingly, based on the cell responses to their environment and on how dopaminergic neurons are recruited after neurodegeneration, the study by Nishimura's group suggests some approaches that can lead to treatments of neurodegenerative diseases such as PD.

Nematodes

The nematode *Caenorhabditis elegans* is ∼1 mm long, free-living, with a short life span (∼3 weeks) and a transparent body wall that allows visualization of all cell types at all stages of development [32]. Its nervous system has 302 neurons [33], including eight dopaminergic neurons in the hermaphrodite form [34,35].

Because these worms can be engineered to express human proteins associated with neurodegeneration, they have been considered appropriate to study PD. PD is characterized by the progressive loss of dopaminergic neurons and accumulation of protein inclusions, mainly of α-synuclein, known as Lewy bodies (LB). Since LB can be considered a histological hallmark of PD, many studies have used *C. elegans* transgenic models to reveal the mechanisms and effects of α-synuclein [20,36,37]. Transgenic *C. elegans* co-expressing α-synuclein and green fluorescence protein (GFP) in dopaminergic neurons were associated with neurodegeneration [36]. This approach has been particularly useful as a model of α-synuclein-mediated neurodegeneration to validate molecular or chemical modulators of α-synuclein discovered in yeast [38,39]. Hardenberg et al. [40] recently reported that a *C. elegans* model of PD helped to understand the relationship between α-synuclein self-assembly and the LB formation. These authors proposed that the formation of LB is associated with the

blocked maturation of α-synuclein condensates in the presence of lipids and possibly other intracellular substances, as also reviewed by [41].

In addition to genetic studies, *C. elegans* has been used to study the effects of pesticides and neurotoxins [42]. Braungart et al. [43] reported that PD-like symptoms can be generated in *C. elegans* by incubating the animals with the neurotoxic agent 1-methyl-4-phenylpyridinium (MPP+), a metabolite of MPTP. Exposure to MPP+ resulted in the death of dopaminergic neurons, reduced mobility, and increased mortality. Masoudi et al. [44] reported that the neurotoxin 6-OHDA generated locomotion defects, tested by a paralysis assay, and loss of dopaminergic neurons fused to GFP. The same study showed that 6-OHDA altered the function of the dopamine active transporter (DAT). Also, Jadiya et al. [37] reported that treatment with 1 mM paraquat modified the expression of heat shock proteins (hsp). Particularly, hsp70 was reduced, possibly affecting cell death, and hsp60 was increased, confirming that paraquat caused oxidative stress. A dose of 4 μM of rotenone impaired the growth of *C. elegans*, caused the loss of dopaminergic neurons, and blocked mitochondrial DNA replication as reported by Zhou et al. [45]

Recently, Sohrabi et al. [46] linked the dysfunction of the branched-chain amino acid transferase 1 (BCAT1) to PD, and reported that RNAi-mediated knockdown of neuronal BCAT1 in *C. elegans* causes abnormal spasm-like 'curling' behavior with age. In a proof-of-concept screen of 50 FDA-approved drugs, the same study identified at least four candidates for potential late-in-life intervention in PD: enasidenib, ethosuximide, metformin, and nitisinone. These findings indicate the utility of the proposed high-throughput platform for automated scoring of worm postures, and in particular, the discovery of potential candidate treatments for PD.

Mollusks

The snail *Lymnaea stagnalis* is a freely moving aquatic mollusk that shows relatively simple behaviors — locomotion, respiration, and feeding — that can be easily quantified and analyzed on a cellular level [47]. One of the most-studied interneurons in the *Lymnaea* central nervous system (CNS) is the dopaminergic giant pedal neuron RPeD1, which is involved in locomotor coordination [48]. Vehovszky et al. [49] reported that rotenone disrupts feeding and locomotion in this snail, and because one possible target of rotenone is the dopaminergic neurons in the CNS, suggested that *L. stagnalis* is a suitable invertebrate model for the study of PD. These authors claimed that the model allows direct analysis of the response of dopaminergic systems to rotenone at the behavioral (reduction in spontaneous locomotion and decreased feeding frequency), cellular [reduction in tyrosine hydroxylase (TH) and dopamine intracellular proportions], and neuronal levels (impairment of dopaminergic inputs of RPeD1).

Dopaminergic neurons, in both vertebrates and invertebrates, have high oxygen consumption and metabolic rates, and generate large quantities of reactive oxygen species (ROS) that can damage mitochondria. Oxidative stress and mitochondrial dysfunction play key roles in the selective destruction of dopaminergic neurons [50,51]. In an ecological approach, Ferreira et al. [52] analyzed the effect of organochlorine pesticides on the oyster *Crassostrea gasar*. Since the study showed protective mechanisms triggered by ROS (including the production of the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase, and glucose-6-phosphate dehydrogenase) [53], oysters also proved to be useful to monitor aquatic biosystems.

Arthropods

Crustaceans

The decapod *Palaemonetes argentinus* is a small South American freshwater shrimp that has been used as an indicator of sites with low environmental quality. Bertrand et al. [54] showed oxidative stress in this shrimp obtained from ecosystems impacted by organic and inorganic pollutants. Since paraquat releases transition metal from storage metalloproteins [55] and triggers the degeneration of dopaminergic neurons in rats [17], *P. argentinus* impacted by pollutants proved to be useful bioindicators in aquatic ecosystems [54]. Behavioral studies have also provided tools to study effects of different chemicals on motor responses in crustaceans [56,57]. However, more-direct studies using these models should be conducted in order to monitor for pesticides.

Using the crayfish *Pacifastacus* as a PD model, Davison et al. [58] reported that the electrophysiological properties of the axons were altered. 6-OHDA (20 mM) affected the axonal permeability, with decreased spike amplitude and enhanced rise time duration. In contrast, the treatment with CAT and SOD promoted axonal

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protection. In order to understand the effects of 6-OHDA in another invertebrate model, the mangrove crab *U. cordatus*, we conducted an *in vitro* study. Following the protocol described in [59], we used cell cultures from the cerebral ganglia of this crab and observed the reduction in the number of TH+ cells in all experimental groups, 1, 3, and 5 days after the exposure to 6-OHDA. During the experimental period, we observed a decrease in the number of TH+ cells in the treatment groups compared with each other (a progressive reduction) and with the controls (Figure 1).

Drosophila

Compared with planarians and *C. elegans*, *Drosophila melanogaster* has a more-developed nervous system, including ∼300 dopaminergic neurons [60]. *D. melanogaster* has emerged as an excellent model organism to study both environmental and genetic factors, providing information on pathways relevant for the PD pathogenesis [61]. Analyses of the functions and mechanisms of the genetic factors associated with PD — such as the encoding α-synuclein for PARK1 [62], PARK4 [63], *LRRK2* (PARK8) [64], *VPS35* (PARK17) [65], and *SYANJ1* (PARK20) [66], and the environmental factors, consisting of oxidative stress-induced toxins, such as MPTP, paraquat, and rotenone [67] — have provided important insights into the disease process. One important conclusion was that these factors cause oxidative damage and mitochondrial dysfunction.

The *Drosophila* model has been also valuable to help to develop new therapeutic strategies. Recent studies have described the neuroprotective effect of ginsenosides, such as Ginsenoside Re [68–70]. The neuroprotective effects of Ginsenoside Re, one of the most active ingredients in ginseng, on a rotenone-induced model of PD, the *Drosophila* brain, were shown and considered the most potent inhibitor of rotenone-induced cytotoxicity [71]. The neuroprotective effects of Ginsenoside Re reduced the rotenone-induced loss of TH+ neurons and partially restored the defects in motor behavior of the *Drosophila* flies. Additionally, the results in [71] suggest that Ginsenoside Re strengthens the cellular antioxidant response by increasing SOD, GPx, and glutathione expression, and reducing the malondialdehyde content.

Another compound tested in *Drosophila* was the poly-methoxyflavonoid, GardeninA. It was shown to have a neuroprotective potential against paraquat-induced parkinsonian symptoms involving reduced survival, mobility defects, and loss of dopaminergic neurons. In addition to being dependent on its antioxidant activities, GardeninA-mediated neuroprotection also involves modulation of the neuroinflammatory and cellular death responses [72].

Ascidians

Ascidians are marine invertebrates that are considered the closest relatives to vertebrates [73]. Dopaminergic neurons have been located in the CNS of larvae of the solitary ascidian *Phallusia mammillata*, in cells related to the otolith, and they have been described as modulators of the onset of metamorphosis in larvae of this

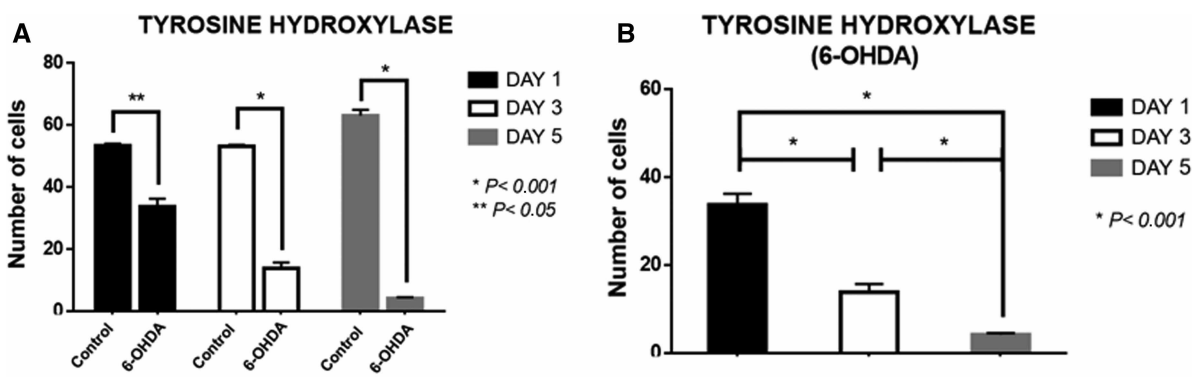

Figure 1. Quantification of tyrosine hydroxylase (TH)+ cells in control and experimental groups (treated with 100 μM of 6-hydroxydopamine, 6-OHDA).

(**A**) comparison of control with experimental groups (1, 3, and 5 days of exposure to 6-OHDA). The results showed reduction in TH+ cells in all experimental groups compared with the controls. (**B**) comparison of treated groups with each other revealed decreases in TH+ cells during the course of the experiment.

species [74]. In another solitary ascidian, *Ciona intestinalis*, transcripts of TH were found in some cells of the left portion of the sensory vesicle of tadpole larvae [75].

Ascidians have been considered appropriate models for studies on factors that protect from toxic effects of pesticides, which can trigger the onset of PD. Zega et al. [76] studied the toxicity of paraquat to *P. mammillata* larvae and compared it to vertebrate organisms. They observed a decrease in dopamine content in the CNS of treated larvae; however, in combined treatments with paraquat and L-ascorbic acid, a common antioxidant, the severity of the malformations was significantly reduced [76]. This result suggests that the oxidative stress is involved in the mechanism of paraquat toxicity to ascidians, similarly to previous descriptions in vertebrates, as recently reviewed by [77].

In the adult, dopaminergic neurons have not been described. However, a molecular study by our group showed that dopaminergic receptors are present in the adult ascidian *Phallusia nigra* [78]. Currently, we are investigating potential effects of rotenone, using adults of another ascidian, *S. plicata*. We conducted a motor behavioral test to analyze the time that ascidians take to close their siphons [79,80]. We injected the animals systemically with 3.0 mg/Kg rotenone, and then stimulated one siphon mechanically from the day of injection until the 10th day, in order to test the time to closure of both siphons. Preliminary results revealed that ascidians took longer to close their siphons two days after the injection of rotenone, compared with day 0, and continued to take longer until the seventh day after rotenone injection. The time to closure of the siphons returned to control levels at the 10th day (Figure 2). To our knowledge, this test has not been used before in PD models, and this is the first study to associate ascidians to parkinsonism using a motor behavior test.

Future directions

Table 1 summarizes reports that used invertebrates as models for PD studies. In this mini-review we focused on some ongoing research using invertebrate models that may provide clues to understand mechanisms of PD, environmental causes, such as those generated by pesticides or chemical use, and certain current therapeutic approaches.

Invertebrates used as animal models for neurodegenerative diseases, such as PD, enable a better understanding of fundamental biological mechanisms. Certain invertebrates (*C. elegans* and *Drosophila*) have demonstrated a high degree of gene and intracellular-pathway homologies with humans and may serve as models for developing novel therapeutic approaches. Invertebrates have both advantages and limits/drawbacks as animal

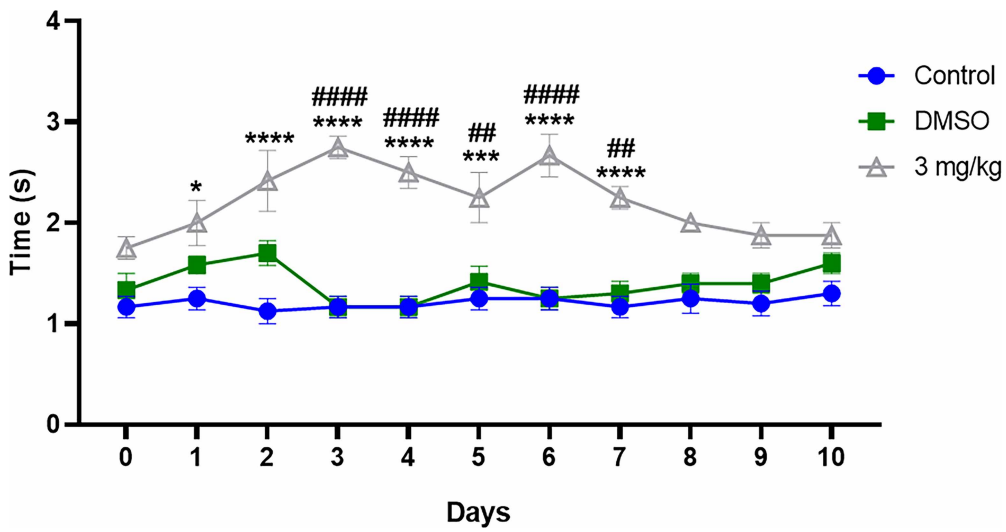

Figure 2. Effect of rotenone on the ascidian *Styela plicata* as evaluated by the siphon test.

After systemic injection with 3 mg/kg of rotenone, the time of closure of the oral siphon after mechanical stimulation was evaluated from days 0 to 10 after injection. (*) animals injected with rotenone versus control animals; (•) control animals; (■) animals injected only with dimethyl sulfoxide (DMSO), the vehicle used to solubilize the rotenone; (Δ) animals injected with 3 mg/kg rotenone. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. (#) rotenone-injected animals versus DMSO-injected animals, ## $P < 0.01$, #### $P < 0.0001$.

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Table 1 Use of invertebrates to model chemically induced parkinsonism-symptoms

Animal model	Chemical used	Study approach
Flatworms	6-OHDA	Immunostaining (with DjTH and AADCA antibodies); Behavioral analysis (negative phototaxis assay); HPLC (quantitative analysis of DA) [31]
Nematodes	MPP+ 6-OHDA Rotenone Paraquat	Locomotion tests (MPP+ decreased the locomotion in a dose-dependent manner); MPP+ affected genetic expression of dopaminergic GFP+ neurons [43] Paralysis assay; Loss of dopaminergic GFP+ neurons; Alteration of DAT function [44] Microscopic analysis (growth impairment and loss of dopaminergic neurons labeled with GPF). qPCR (blocked mitochondrial DNA replication) [45] qPCR (modified the expression of hsp-70 and hsp-60) [37]
Mollusks	Rotenone	Behavioral tests (locomotion assay and feeding behavior); Electrophysiological experiments (to determine intracellular activity of dopaminergic neurons from pedal ganglia); HPLC (quantify dopamine); Immunocytochemistry (TH and dopamine) [49]
Crustaceans	6-OHDA	Electrophysiological tests of the nerve cord (6-OHDA reduced spike amplitude, increased axonal permeability, and enhanced rise time and duration of action potential); The enzymes SOD and catalase restored neuronal excitability [58]
Drosophila	Rotenone, Paraquat, Ginsenoside Re (neuroprotective effects)	Longevity and survival tests; Locomotion activity (climb); Biochemical parameters (total protein determination); Analysis of toxicity [catalase, hydrogen-peroxide level, nitric-oxide (nitrate/nitrite) level]; Acetylcholinesterase activity; Malondialdehyde level; Protein carbonyl content; Cell viability (mitochondrial metabolic rate); Molecular docking [81] Mobility assay; Immunofluorescence analysis (and GFP-positive dopaminergic neurons); Quantitative real-time RT-PCR (expression of Relish, the human NFκB orthologue); Nitric-oxide analysis; Lipid-peroxidation assay; UPLC-QTOF-MS SIR analysis of *Drosophila* head extracts; Caspase-3 activity [72] Cell viability (MTT based); Cell apoptosis (Annexin V); Nitrite concentration;TNF-α and IL-6 concentrations; ROS levels (CM-H2DCFDA); Western-blot analysis [68] Behavioral tests (climb setup, flying tests, gait analysis) [82]
Ascidians	Paraquat	Morphological analysis of *Phallusia mammillata* larvae; Immunohistochemistry of β-tubulin, dopamine, and GABA; *In situ* hybridization (expression of TH) [76]

AADCA, Aromatic Amino Acid Decarboxylase; CM-H2DCFDA, General Oxidative Stress Indicator; DA, Dopamine; DAT, Dopamine Active Transporter; DjTH, *Dugesia. japonica* Tyrosine Hydroxylase; GABA, Gamma Aminobutyric Acid; GFP, Green Fluorescent Protein; HPLC, High Performance Liquid Chromatography; hsp, Heat-Shock Proteins; IL-6, Interleukin-6; MPP+, 1-methyl-4-phenylpyridinium; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NFκB, Nuclear Factor Kappa B; NO, Nitric Oxide; qPCR, Quantitative Polymerase Chain Reaction; RT-PCR, Real-time Polymerase Chain Reaction; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase; TH, Tyrosine Hydroxylase; TNF-α, Tumor Necrosis Factor-α; UPLC-QTOF-MS SIR, Ultra-performance Liquid Chromatography Quadrupole Time-of-flight Mass Spectrometry Selected Ion Recording; 6-OHDA, 6-hydroxydopamine.

models for diseases, including but not limited to PD (Figure 3). Readers must keep in mind that it is not possible to model all diseases using invertebrates, because they do not have all corresponding human genes and similar organs to humans. Another drawback is the body constitution of invertebrates, since some have cuticles that act as a physical barrier, limiting pharmacokinetic approaches (absorption, distribution, and metabolism) to using them as models for investigating human diseases.

Invertebrates and vertebrates share some molecules, although certain molecules may not be involved in the same functions. Dopamine is the key molecule for responses, such as food reward and motor functions in

	Advantages	Drawbacks
Flatworms 	• Planarians can regenerate any body part, even the CNS, from small pieces within a few days [83] • Planarians are easy and cost-effective to maintain, and can be grown to large populations [83] • Many planarian proteins are significantly similar to human proteins [83] • The genome of *Schmidtea mediterranea* has been sequenced; transcriptome datasets are being generated to facilitate understanding of the genetic regulation of planarian regeneration [83] • The presence of dopaminergic neurons may be useful to study effects of drugs and neuroprotective factors [84]	• Few studies and techniques to understand the genetic and metabolic pathways involved in PD [85] • Difficult to comprehend the acute and chronic conditions involving neurodegeneration, especially because of the limits of techniques [85] • Incomplete knowledge of the homology between the metabolic and genetic pathways in mammals (specifically humans) and planarians [85]
Nematodes 	• Simple nervous system with 8 dopaminergic neurons [86] • Conservation of basic biological processes and genes related to PD • May exhibit some key features and phenotypes of PD • Short life cycle of approximately 3–5 days [87]	• Lack of some PD-related genes and complex protein families [87]
Mollusks 	• Significant experimental efficiency [88] • Shorter time needed for experiments [88] • Low cost of care [88] • Functional and regulatory elements in the genome are the basis of metabolic pathways and processes, especially in humans [88] • Dopaminergic neuron involved in muscular locomotion [48]	• Some genes related to PD, such as alpha-synuclein, were not reported, impeding the study of specific proteins
Crustaceans 	• Ease of maintenance and low cost [25] • CNS and neural cells well described [89, 90] • Presence of dopamine and TH in nerve cells • Evolution and ecological model - decapod crabs represent a transition between aquatic and terrestrial habitats [91, 92] • Serve as a bioindicator of pollution with metals that can induce parkinsonism in humans [93]	• Collection from the wild restricted to specific seasons of the year • Lack of behavioral tests conducted in crustacean PD models • In crabs, the carapace and open circulatory system impede in-vivo study involving injection of chemicals • Small cerebral ganglion requires serial sections less than 5 µm thick
Drosophila 	• Complex nervous system with dopaminergic neurons in clusters (groups of cells) [94] • Dopaminergic neurons are detectable in both larval and adult stages • Dopamine synthesis pathways are similar to humans' • Short life cycle • Conservation of basic biological processes and genes related to PD [87]	• Limitations in reproducing the complex features of PD • Lack of some PD-related genes and complex protein families [87]
Ascidians 	• Phylogenetic proximity to vertebrates, thus sharing characteristics of their body plan with vertebrates, turning them suitable models for comparative studies of regeneration and neurodegenerative diseases [95] • The organization of the ganglionar cortex (CNS) of *Styela plicata* is more similar to the brain of vertebrates than to the CNS of other invertebrates [79] • Low maintenance cost in aquaria, easy maintenance and easy to obtain • Regeneration of the CNS [96]	• Absence of some genes related to PD, such as alpha-synuclein, which prevents studying this protein in this animal model • Lack of neuronal complexity compared to vertebrates • They are sessile animals

Figure 3. Groups of invertebrates used as models to study parkinsonism.

many animal groups. One exception is the arthropods, in which the molecule related to reward is the biogenic amine octopamine [97]. Therefore, focusing on different invertebrate models may help to understand, from an evolutionary point of view, the role of key molecules in different neurological human diseases such as PD.

While mammals, including humans, are not capable of regenerating their CNS after mechanical, chemical, or pathological damage such as occurs in neurological diseases, invertebrates have a considerable ability to restore their nervous system [26,80,96,98]. Neuroregeneration mechanisms are under investigation, and the increase in knowledge of the mechanisms underlying this process may help to understand the roles of cells, molecules and genes, which could be used to develop molecular targets to treat PD in the future.

Perspectives

- Currently, invertebrate models are being used to understand the pathophysiology of PD. This research may have implications for humans and suggest directions of future studies.
- Less frequently used to study PD, invertebrates as a group have several advantages, such as short lifespans, ease of maintenance and manipulation, low cost, and genetic homology with vertebrates.
- Because invertebrates have several advantages, invertebrate models can provide clues to develop new therapeutic targets for PD.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

6-OHDA, 6-hydroxydopamine; BCAT1, branched-chain amino acid transferase 1; CAT, catalase; CNS, central nervous system; DAT, dopamine active transporter; GFP, green fluorescent protein; GPx, glutathione peroxidase; hsp, heat shock proteins; LB, Lewy bodies; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; *PARK*, PD genes; PD, Parkinson's disease; ROS, reactive oxygen species; RPeD1, dopaminergic giant pedal neuron; SOD, superoxide dismutase; TH, tyrosine hydroxylase.

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