PHYSICAL ACTIVITY–INDUCED NEUROPROTECTION IN PARKINSON’S DISEASE IS MEDIATED BY NEUROTROPHIC FACTORS

Hunter Twedt

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PHYSICAL ACTIVITY–INDUCED NEUROPROTECTION IN PARKINSON’S DISEASE IS MEDIATED BY NEUROTROPHIC FACTORS

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SPRING 2020
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ABSTRACT

Parkinson’s disease (PD) is a progressive neurological movement disorder manifested by motor and non-motor disturbances that are brought about by degeneration of dopaminergic (DAergic) neurons in the substantia nigra (SN), causing a decline in dopamine (DA) levels in the striatum. Pharmacological and surgical treatment of PD are often ineffective, cause severe side effects, and the benefits seen decrease with disease progression; therefore, there is a need to seek new therapies. Physical activity (PA), which includes exercise, is able to improve many systems in the body, including motor and cognitive function, and may serve as a viable option for remedy of PD. Due to many limitations, human studies are unlikely to explain whether such intervention may be neuroprotective in PD patients; therefore, the use of animal models is imperative. PA in animal models of PD has neuroprotective effects on DAergic neurons and current hypotheses infer a central role of neurotrophic factors (NTFs); however, the exact mechanisms of their action have yet to be elucidated. In the present review, studies will be discussed that highlight how PA increases NTF mobilization leading to protection of DAergic neurons and improved motor performance. Furthermore, this review will explore NTF influence in several postulated aberrant cellular processes where intervention could be made to treat PD, including neuroplasticity and synaptic transmission, angiogenesis and vasodilation, mitochondrial dysfunction through oxidative stress and protein aggregation, and neuroinflammation.

INTRODUCTION

PD was first described in 1817 by James Parkinson, described as a “Shaking Palsy”, and eventually went on to bear his name [1]. PD is the commonest neurodegenerative disease after Alzheimer’s disease, affecting roughly 1% of adults over the age of 60 [2] and is currently the 14th leading cause of death in the United States [3]. PD is a progressive neurological disorder that is characterized by depigmentation of the SN caused by the selective loss of DAergic neurons and the presence of misfolded protein aggregates known as Lewy Bodies (LBs) [4].

Because it is a progressive disease, symptoms generally develop gradually and can be classified as motor and non-motor. Motor symptoms are usually used to diagnose PD and commonly include tremor, bradykinesia (slowness of movement), stiffness, and postural instability [3]. Non-motor symptoms may coincide with PD or may appear before the diagnosis is made (pre-motor symptoms) [5]. Non-motor symptoms include, but are not limited to, lack of emotional involvement and interest (apathy), inability to experience pleasure from activities that were otherwise found enjoyable (anhedonia), memory problems, loss of smell and taste, excessive sweating, constipation or urinary problems, depression or mood disturbances, skin problems, daytime drowsiness, and disruptions in sleep [5,6,7].

The cause of PD, while still unknown, is likely multifactorial. It was originally thought that environmental factors were the predominant cause of PD, especially after the influenza outbreak of 1918, when a group of affected individuals developed postencephalitic Parkinsonism [8]. The environmental hypothesis was supported further when four individuals developed Parkinsonism after administering an illicit drug intravenously – later discovered to be primarily 1-methyl-4-
phenyl-1,2,5,6-tetrahydropyridine (MPTP) with smaller amounts of 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP) – in the early 1980s [9]. Since this time, a number of environmental factors have been implicated as a cause of PD, including metals, pesticides, farming or rural life, industrial toxins, high caloric intake, and head trauma [10,11]. Although a large part of the 1900s focused on the environmental factors that lead to PD, the current knowledge on the underlying causes of PD has changed greatly. A large percentage of patients (>85%) with late-onset or idiopathic PD do not seem to have inherited the disease, but a family history of PD puts an individual at high risk, as some studies have shown large families with evident Mendelian-pattern inherited PD (<10%) [12]. While the etiology remains elusive, several mechanisms, including oxidative stress, mitochondrial dysfunction, disrupted proteolysis, neuroinflammation, and excitotoxicity are involved in the pathophysiology of the disease leading to neuronal cell death [13,14,15].

Pharmacological and surgical treatments are current therapies for PD which can help many patients, but benefits are limited. Pharmacological treatments, the current gold standard, are largely based on DA replacement such as DA precursors (levodopa, L-DOPA), DA agonists (amantadine, apomorphine), MAOB inhibitors (selegiline, rasagiline) [16]. Other potential drug treatments include COMT inhibitors (entacapone, tolcapone), and A2A antagonists (caffeine, tozadenant) [17]. When medication cannot treat the severity of PD symptoms, deep brain stimulation is a surgery that sends electrical signals to the SN. Stimulation of the SN and surrounding areas (e.g., globus pallidus, subthalamic nucleus, caudal zona incerta) has shown to treat motor symptoms dyskinesia and tremor through a mechanism of neurogenesis and neuroplasticity, which is likely to improve long-term symptoms [16,18]. Although, short-term, various medical and surgical therapies alleviate symptoms of PD, there is a large list of adverse effects (Table 1) [19] and, to date, there are no established disease progression-modifying or neuroprotective therapies [20].

<table>
<thead>
<tr>
<th>Side effects and adverse reactions from current PD treatments</th>
<th>Surgical intervention</th>
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<td>Dopaminergic drugs</td>
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<tr>
<td>Dopamine dysregulation syndrome</td>
<td>Dopamine dysregulation syndrome</td>
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</tbody>
</table>

The presymptomatic phase of PD is estimated to be 10-20+ years [21], which allows for the potential to test non-pharmacological interventions. PA may serve as a non-invasive, non-pharmacological treatment of PD that has no side effects yet has proven benefits for multiple organ systems including the central nervous system (CNS) [22,23,24]. Recent studies display that regular PA, such as aerobic exercise, strength training, and flexibility can enhance brain plasticity –
playing a vital role in the improvement of motor and cognitive function [25]. Although a vast amount of evidence supports that PA could be used as a neuroprotective agent to combat PD, the mechanism underlying the diminished risk is still not fully understood [26] and is difficult to study in a human model.

Studies in rodents that have been subjected to PA around the time of application of neural insult, to simulate PD, have shown much promise to elucidate the etiology of PA-induced neuroprotection. Neurotoxic agents that are most often used for this purpose include MPTP and its active metabolite 1-methyl-4-phenylpyridine (MPP+), 6-hydroxydopamine (6-OHDA), and lipopolysaccharide (LPS). Many of these studies have found that exercise, a subcategory of PA, reduced the loss of DAergic neurons in the SN through a neuroprotective mechanism involving upregulation of NTFs.

NTFs are peptides required for the maintenance, survival, specification, and maturation of specific neuronal populations [27]. Through a widely renowned process known as the “neurotrophic factor hypothesis”, neurons compete for a limited supply of these peptides during development, which are produced by target tissues; neurons that fail to receive an adequate supply perish by means of programmed cell death, known as apoptosis [28]. In the post-developmental, mature organism, NTFs are essential for maintaining neuronal functionality and their specific phenotype [29]. NTFs have also been shown to have the means of modifying neuronal dysfunction, inflammatory reactions, and neural plasticity during periods of pathological states such as neurodegeneration or aging [29].

More than 20 different NTFs have been identified and fall into one of four families of related molecules: (i) the neurotrophin family; (ii) the glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFLs); (iii) the neurotrophic cytokines, or “neurokines”; and (iv) the newer, unconventional, cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) [27,30]. This review will focus primarily on NTFs that have been extensively studied in animal models and are known to act through pathways that are disrupted in PD. The commonest being BDNF, GDNF, VEGF, and IGF-1.

Up to this point, an overwhelming amount of evidence supports PA as an effective prevention or therapy for PD; however, the mechanisms underlying these claims are not fully understood [26]. While this review will give a short background and discussion of several signaling cascades and cellular mechanisms, it is not meant to take the place of a more detailed review on each specific topic covered. This goal of this article is to analyze papers based on studies using animal models of PD in order to investigate the effect of PA on several mechanisms that have been proposed to explain the effects of PA on neuroprotection in PD, centered around NTF action. These include increased NTF synthesis and release, protection of DAergic neurons and improved motor function, enhanced neuroplasticity and synaptic transmission, increased angiogenesis and vasodilation, improved mitochondrial function through resistance to oxidative stress and reduced protein misfolding/aggregation, and reduced neuroinflammation.
## STUDIES ON ANIMAL MODELS

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal Model / Treatment</th>
<th>PA Type</th>
<th>PA Start Timing, Duration</th>
<th>PA Parameters</th>
<th>NTF Analysis</th>
<th>DA/TH Analysis</th>
<th>Motor Function Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aguiar et al., 2016 [31]</td>
<td>Mouse / 6-OHDA</td>
<td>Treadmill</td>
<td>6 wks after 6-OHDA</td>
<td>16 m/min, increased by 2 m/min every 3 min until exhaustion; 5 d/wk</td>
<td>NA</td>
<td>+</td>
<td>+</td>
</tr>
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<td>Muhammed et al., 2010 [32]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>4 wks after MPTP</td>
<td>18 m/min for 40 min/d; 5 d/wk</td>
<td>+</td>
<td>+</td>
<td>NA</td>
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<tr>
<td>Cohen et al., 2003 [33]</td>
<td>Rat / 6-OHDA</td>
<td>Forced limb use</td>
<td>up to 4 wks after 6-OHDA</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Garcia et al., 2017 [34]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>1 mo before 6-OHDA</td>
<td>3 d/wk</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Gerecke et al., 2010 [35]</td>
<td>Mouse / MPTP</td>
<td>Running wheel</td>
<td>3 mo before MPTP</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Gerecke et al., 2012 [36]</td>
<td>Mouse / MPTP</td>
<td>Running wheel</td>
<td>3 mo before MPTP</td>
<td>4.8 km/d</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Goes et al., 2014 [37]</td>
<td>Mouse / 6-OHDA</td>
<td>Swimmin g exercise</td>
<td>4 wks after 6-OHDA</td>
<td>2% body weight attached to tail; 5 d/wk</td>
<td>NA</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hashemvarzi et al., 2017 [38]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>4 wks before 6-OHDA</td>
<td>15 m/min for 30 min/d; 5 d/wk</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Jang et al., 2017 [39]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>8 wks after MPTP</td>
<td>10 m/min for 60 min/d; 5 d/wk</td>
<td>NA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Koo et al., 2017 [40]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>8 wks after MPTP</td>
<td>10 m/min for 40 min/d; 5 d/wk</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Koo et al., 2017 [41]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>8 wks after MPTP</td>
<td>10 m/min for 60 min/d; 5 d/wk</td>
<td>NA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lau et al., 2011 [42]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>1 wk before, 5 wks during, 12 wks after MPTP</td>
<td>Up to 15 m/min for 40 min/d; 5 d/wk</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Treatment</td>
<td>Duration</td>
<td>Exercise Protocol</td>
<td>Outcomes</td>
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<tr>
<td>Mohammadi et al., 2019 [43]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>4 wks after 6-OHDA</td>
<td>15 m/min for 30 min (mild) or up to 120 min (progressive); 5 d/wk</td>
<td>+</td>
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<tr>
<td>Real et al., 2013 [44]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>4 wks after 6-OHDA</td>
<td>10 m/min for 40 min/d; 3 d/wk</td>
<td>+</td>
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<tr>
<td>Real et al., 2017 [45]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>4 wks before 6-OHDA</td>
<td>3 d/wk</td>
<td>NA</td>
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<tr>
<td>Shi et al., 2017 [46]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>4 wks after 6-OHDA</td>
<td>11 m/min for 30 min/d; 5 d/wk</td>
<td>NA</td>
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<tr>
<td>Shin et al., 2016 [47]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>2 wks after MPTP</td>
<td>8 m/min for 30 min/d; 5 d/wk</td>
<td>NA</td>
<td></td>
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<tr>
<td>Smeine et al., 2015 [48]</td>
<td>Mouse / MPTP</td>
<td>Running wheel</td>
<td>3 mo before MPTP</td>
<td>NA</td>
<td>NA</td>
<td></td>
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<tr>
<td>Sung et al., 2012 [49]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>4 wks after MPTP</td>
<td>12 m/min for 30 min/d; 5 d/wk</td>
<td>NA</td>
<td></td>
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<tr>
<td>Tajiri et al., 2010 [50]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>4 wks after 6-OHDA</td>
<td>11 m/min for 30 min; 5 d/wk</td>
<td>+</td>
<td></td>
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<tr>
<td>Tillerson et al., 2001 [51]</td>
<td>Rat / 6-OHDA</td>
<td>Forced limb use</td>
<td>up to 4 wks after 6-OHDA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Tillerson et al., 2002 [52]</td>
<td>Rat / 6-OHDA</td>
<td>Forced limb use</td>
<td>up to 4 wks after 6-OHDA</td>
<td>NA</td>
<td>NA</td>
<td></td>
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</tr>
<tr>
<td>Tillerson et al., 2003 [53]</td>
<td>Rat / 6-OHDA and MPTP</td>
<td>Treadmill</td>
<td>up to 4 wks after 6-OHDA/MPTP</td>
<td>15 m/min for 30 min/d</td>
<td>NA</td>
<td></td>
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</tr>
<tr>
<td>Tuon et al., 2012 [54]</td>
<td>Rat / 6-OHDA</td>
<td>Treadmill</td>
<td>8 wks before 6-OHDA</td>
<td>13-17 m/min for 25 min/d; 3 or 4 d/wk</td>
<td>+</td>
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<tr>
<td>Toy et al., 2013 [55]</td>
<td>Mouse / MPTP</td>
<td>Treadmill</td>
<td>6 wks after MPTP</td>
<td>10-24 m/min; 5 d/wk</td>
<td>NA</td>
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<tr>
<td>Tsou et al., 2015 [56]</td>
<td>Rat / MPP+</td>
<td>Treadmill</td>
<td>4 wks before MPP+</td>
<td>12-15 m/min for 60 min/d; 5 d/wk</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al., 2011 [57]</td>
<td>Mouse / LPS</td>
<td>Treadmill</td>
<td>4 wks before LPS</td>
<td>10 m/min for 60 min/d; 5 d/wk</td>
<td>+</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. A simplified overview of studies which subjected rodent animal models to some form of PA with a PD lesion displaying the compound used for neurotoxic insult, form of PA, PA start timing with respect to the PD lesion, duration of PA, and analysis of NTF, DA/TH, and motor function. “+” = an improvement or increase, NA = not examined in the study.

Table 2. A simplified overview of studies which subjected rodent animal models to some form of PA with a PD lesion displaying the compound used for neurotoxic insult, form of PA, PA start timing with respect to the PD lesion, duration of PA, and analysis of NTF, DA/TH, and motor function. “+” = an improvement or increase, NA = not examined in the study.

<table>
<thead>
<tr>
<th>Yoon et al., 2007 [58]</th>
<th>Rat / 6-OHDA</th>
<th>Treadmill</th>
<th>2 wks after 6-OHDA</th>
<th>2 m/min for 5 min, 3 m/min for 25 min; 30 min/d</th>
<th>NA</th>
<th>+</th>
<th>+</th>
</tr>
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</table>

**PHYSICAL ACTIVITY INCREASES NEUROTROPHIC FACTOR SYNTHESIS AND RELEASE**

Table 2 gives a simplified overview of numerous studies that show positive effects of PA on motor performance, NTF mobilization, and protection of DA neurons in neurotoxin-induced rodent PD models. The exact mechanism by which PA increases the mobilization of NTFs is complex and not yet fully understood. However, it has been shown that PA is accompanied with increased metabolism, due to a larger energy requirement. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is one of many transcriptional regulators that acts as a metabolic sensor and alters gene expression in response to metabolic changes [59]. With PA in mice, Steiner et al [60]. found that PGC-1α is overexpressed in the brain and, through activation by silent information regulator 1 (SIRT1), induces synthesis of mitochondria (i.e., mitochondrial biogenesis).

Following mitochondrial biogenesis, PGC-1α has also been shown to increase oxidative phosphorylation, and thus, energy production [61]. Metabolic increases, such as those seen with PA, confer a greater amount of circulating energy metabolites and intermediates; especially ketone bodies like D-β-hydroxybutyrate (DBHB) and acetoacetate, derived from acetyl-CoA. Several of these metabolites have shown an ability to circulate throughout the body and serve as an energy source for several tissues, including the brain by crossing the blood brain barrier (BBB). These endogenous energy metabolites are capable of inducing promoters for the genes of NTFs, augmenting their synthesis and release (Fig 1). For example, in mice subject to voluntary running for 4 weeks, researchers found increased levels of DBHB, which led to amplified BDNF expression [62].

![Figure 1. Physical activity increases the mobilization of NTFs.](image)

**Figure 1. Physical activity increases the mobilization of NTFs.**

(A) Representative schematic displaying PA-induced enhancements in metabolism increases the mobilization of high-energy metabolic intermediates in the brain, or traverse the bloodstream and act in the brain, to increase synthesis and release of NTFs. This is exemplified at a study by Lau et al [42], where, in an exercised PD model, there are increases of (B) BDNF and (C) GDNF in the SN and striatum.
Although PGC-1α mainly resides within mitochondria to regulate several of its functions, Safdar et al. [63] found that PGC-1α translocates from between mitochondria and the nucleus to facilitate its action. While it has been known that PGC-1α binds to promoters of nuclear-encoded mitochondrial genes [64], it is entirely plausible that PGC-1α, itself, acts as a signal of increased energy production, giving rise to increased NTF production to promote transcellular signaling for survival of larger populations of neurons in the CNS. It is apparent that there are multiple mechanisms taking part in the PA-induced NTF expression, yet enhanced metabolism leading to increased energy production and levels of metabolic intermediates displays one possible explanation for increased NTF expression and mobilization.

**PHYSICAL ACTIVITY PROTECTS DOPAMINERGIC NEURONS**

Many of the studies suggest that exercise can promote neuroprotection, through the upregulation of NTFs, against neurotoxin treatment. Survival of DAergic neurons is mediated by transcriptional regulation of the tyrosine hydroxylase (TH) gene that is involved in DA metabolism. TH is an enzyme that converts tyrosine to L-dihydroxyphenylalanine (L-DOPA), which is then converted to DA by the L-amino acid decarboxylase (AADC) aromatic enzyme. In addition to DA, several of its metabolites, including 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) can be used to report DA concentrations as a measure of DAergic function and survival. TH levels can also be used as a marker of DAergic neurons, due to the finding that increased TH activity promotes their survival [65]. In the proceeding text in this section, several studies will be summarized to exemplify exercise-induced protection of DAergic neurons and improved motor performance of PD-lesioned exercised (PDE) compared to PD-lesioned sedentary (PDS) animals.

Protection of DAergic neurons was displayed in two studies by Gerecke et al. with voluntary running wheel exercise prior to MPTP treatment in mouse PD models. The first study [35] investigated the critical duration of exercise necessary for neuroprotection of DAergic neurons and found that 1 month provided no neuroprotection, 2 months showed partial protection, and 3 months of exercise completely protected DAergic neurons in the SN from subsequent neurotoxin treatment. The second study [36] used this data and subjected mice to 90 days (3 months) of voluntary exercise with or without a knockout in the BDNF gene, to test NTF-induced neuroprotection from exercise. Results showed that 90 days of unrestricted running in wild-type mice completely protected against MPTP-induced neurotoxicity, while mice with a knocked out BDNF gene were not protected from MPTP-induced DAergic neuron loss in the SN. Lastly, Wu et al. [57] showed that LPS-treated mice had a reduction in the number of DAergic neurons and BDNF levels in the SN. They then showed that 4 weeks of treadmill exercise prior to LPS injection prevented the loss of DAergic neurons, and elevated DA and DOPAC in the SN and striatum. The critical role that BDNF played in the exercise-induced neuroprotection was highlighted by their findings that blocking the BDNF receptor, and thus its action, abolished protection against DAergic neuron loss.
Several studies also display effects of exercise during neurotoxic insult. Lau et al. [42] used a program that consisted of treadmill exercise 1 week prior, 5 weeks during, and 12 weeks after MPTP hydrochloride + probenecid treatment in a mouse PD model. Results showed a significant increase in the number of DAergic neurons in the SN when compared to PDS mice (Fig 2). Additionally, increases in levels of TH, DA, and dopamine active transporter (DAT) were seen in PDE mice. With a NTF analysis, researchers discovered elevated levels of BDNF and GDNF in the striatum and SN of PDE mice; importantly, NTF levels in the PDE group were higher than both the PDS and non-PD controls. Similarly, Tuon et al. [54] investigated the effects of incremental treadmill exercise 8 weeks after 6-OHDA treatment and found striatal increases of 59% for TH and 33% for BDNF, in PDE rats when compared to PDS.

A larger number of studies compiled have monitored the neuroregenerative effects of exercise when the training began after neurotoxic insult. Koo et al [40]. subjected mice to 8 weeks of treadmill exercise after an MPTP lesion and showed inhibition of DAergic neuron loss in the SN by promotion of TH and DAT. Real et al [44]. exercised rats for 4 weeks after injection of 6-OHDA and found rescue of TH and BDNF levels in the SN of PDE compared to PDS groups. They, too, validated the action of BDNF involvement in neuroprotection by showing that a BDNF receptor blockade reversed the neuroprotective effects of exercise. Cohen et al [33]. tested forced limb use by placing a cast on the rat forelimb for 7 days following 6-OHDA administration to the medial forebrain bundle (MFB). The cast was placed on the forelimb ipsilateral to the injected MFB, forcing the animal to use the contralateral limb, in which motor function was negatively affected by the 6-OHDA injection. Casted animals (PDE) were found to have higher levels of GDNF in the striatum corresponding to the overworked limb compared to non-casted animals (PDS). Additionally, the PDE group displayed attenuated striatal DA and DOPAC loss compared to PDS.
Tajiri et al [50]. examined the effects of treadmill exercise for 4 weeks following a 6-OHDA lesion on DA neurons and NTFs. Results showed a preservation of DAergic-fibers in the striatum and DAergic-neurons in the SN for PDE compared to the PDS group. Furthermore, they found that both BDNF and GDNF increased in control- and 6-OHDA-treated sides of the striatum in the PDE group. Lastly, Mohammadi et al [43]. tested mild vs progressive treadmill exercise for 4 weeks in rats following a 6-OHDA lesion. They found that with progressive treadmill exercise, but not mild, there were increases in both DA and TH in the striatum. Additionally, to a larger degree in progressive exercise, there were increases in striatal NTFs: MANF, CDN, and NGF.

PHYSICAL ACTIVITY IMPROVES MOTOR PERFORMANCE

In order to assess motor function, several tests were carried out across various studies. The most used was the rotational behavior test (Fig 3a,b), which has become a mainstay for testing the extent of motor impairment from loss of nigrostriatal or mesolimbic DAergic neurons due to neurotoxic insult. The extent that various authors are concerned with this test is its ability to demonstrate rescue of DAergic neurons from cell death.

In this test, rodents are placed in a rotameter, a bowl-like apparatus with a flat center and steep walls, and injected with amphetamine or apomorphine to increase motor activity. In control rodents (those not lesioned), the animal exhibits exploratory behavior, but not high numbers of rotational movements. Lesioned animals, however, display profound asymmetrical rotations, i.e., often turning in one direction over the other, in the forelimb contralateral to the lesion. This model is useful in that it allows for assessment of DA neuron loss and functional changes that results (impairment or recovery) on the lesioned side in direct comparison to the non-lesioned, intact side [66,67].

In the studies used throughout this review as evidence of exercised-induced neuroprotection, 10 of them [31,33,38,43,44,45,46,52,54,58] performed the rotation test and all 10 of them showed a positive result of exercise therapy, where neurotoxin-induced PD rodents
displayed a significant decrease in the number of asymmetrical rotations (Fig 3c). In the studies that showed this positive result of exercise, only 5 of them [33,38,43,44,54] concurrently monitored and studied the effects of NTF action; therefore, it becomes imperative to examine NTF action at the cellular level in order to elucidate the beneficial effects of NTF action in animal models of PD.

**MOLECULAR MECHANISMS OF NEUROTROPHIC FACTOR ACTION**

In the early 1990’s it was known that PA played roles in improving neuronal survival in response to brain insult by promoting neuronal vascularization and stimulating neurogenesis. Since that time, it has been discovered that many of these beneficial aspects are due to direct action on molecular machinery in the CNS itself, rather than through improvements in general health [69]. The preceding sections summarized how PA attenuated the reduction of DAergic neurons and mobilized NTFs to provide motor improvements in animal neurotoxin-induced models of PD. Now this review will discuss the possible underlying molecular mechanisms of these findings. Although these mechanisms are not all well-established, large amounts of evidence suggest that NTFs are the key mediators of the beneficial effect of PA [70] and, due to an overwhelming amount of literature on the cellular effects of various NTFs, a large portion of this section will be dedicated to those that have been highly implicated in PD.

**BDNF**

BDNF has roles in neuronal and glial development, neuroprotection, and synaptic interactions which are critical for cognition and memory [71]. It is likely the most highly studied NTF for its role in PD, and has been shown to promote the survival, function, and differentiation of SN DAergic neurons [72]. ProBDNF, the BDNF precursor, undergoes proteolytic cleavage to form mature BDNF, which acts through a high affinity tyrosine receptor kinase B receptor (TrkB) on cell surfaces, leading to the activation of downstream MAPK, PI3K/Akt, and PLCγ pathways [73,74]. In a study that investigated the effects of PA on depressive symptoms in PD, Tuon et al [75] found a significant decrease in striatal levels of proBDNF, BDNF, and TrkB upon treatment with 6-OHDA; however, with PA, there was a reversal of the diminished expression and prevention in depressive-like behavior. Thus, researchers proposed that the beneficial effects of PA were likely a result of increased maturation of proBDNF to BDNF.

Using in situ hybridization, high expression levels of BDNF are apparent in many midbrain structures and the cerebellum, with a lower expression level in the striatum [76]. BDNF expression is particularly high in the SN, but is severely attenuated in PD. Parain et al [77] presented this by detecting that 65% of all melanin-containing neurons in the SN were immunoreactive for BDNF while, in PD patients, only 9.6% of these pigmented neurons were reactive for BDNF. Additionally, previous studies suggest that exogenous BDNF administration can increase the survival and/or aid in the recovery of injured DAergic neurons when treated with MPP+, with or without BDNF as a potential therapeutic agent [78].
In cultured hippocampal neurons it was shown that there is a rapid increase of TrkB expression after BDNF treatment followed by a reduction after several days through a process of transcriptional regulation [79]. Neuronal activity, which PA is known to boost, generates a sustained mode of activation for TrkB and its downstream signals, leading to attenuation of neuronal cell death and amplified dendritic branching [74]. In both non-pathological and pathological conditions, both proBDNF and BDNF are secreted, but have opposite effects. ProBDNF preferentially binds the pan-neurotrophin receptor (p75NTR) [80] while mature BDNF binds specifically to TrkB receptors, promoting cell survival and increases spine complexity [81,82].

**GDNF**

GDNF has been shown to play a significant role in the development and survival of midbrain DAergic neurons as well as spinal motor neurons via its neuroprotective role of reducing neuronal atrophy that coincides with age-related deficits [83]. It is crucial for the maintenance of neuronal morphological and neurochemical phenotypes and protects DAergic neurons from toxic damage [84]. It has been shown that GDNF is approximately 5-10 times more potent than BDNF in promoting DAergic neuron survival [85]. GDNF acts through the receptor tyrosine kinase rearranged during transcription (Ret), but the activation of Ret requires association with the membrane-bound protein GDNF family receptor α (GFRα) [86]. Formation of the Ret-GFRα complex induces activation of downstream signaling including, MAPK, PI3K/Akt, and PLCγ pathways [87].

In a study by Boger et al., a heterozygous partial deletion of the *Gfra1* gene in aging mice (26 months) showed a decrease in DAergic fiber density in the striatum accompanied by a lower number of DAergic neurons in the SN, and an increased sensitivity of nigrostriatal DAergic neurons to MPTP toxicity [88], suggesting a pivotal role of GFRα in trophic neuroprotection by GDNF signaling. As a NTF, GDNF promotes the DA phenotype of DAergic neurons and may, in this way, exert some of its neuroprotective actions. Through its action, GDNF increases transcription factors (Nurr1, Pitx3) that participate in the expression of genes involved in DA metabolism including TH, DAT, AADC, and vesicular monoamine transporter (VMAT) [89].

**VEGF**

VEGF was originally believed to be a factor that played regulatory roles in vascular growth and development [90]; in more recent times, it has also been shown to have direct action on neuronal populations such as, stimulating axonal growth and guidance [91], stimulating neurogenesis [92], regulating neuronal migration [93], and promoting dendrite patterning and synaptic plasticity [94]. The VEGFs are a family of growth factors that include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor [95]. The biological activity of the VEGFs is mediated through the binding to two classes of receptor. The first being VEGF tyrosine kinase receptors 1-3 (VEGFR1-3), and the second, non-tyrosine kinase receptors, being neuropilin (NP-1, NP-2) which function as co-receptors for the VEGFRs [96]. Upon ligand binding, VEGFR
undergoes autophosphorylation, activating intracellular signaling pathways including PI3K/Akt and MAPK cascades, which have been shown to protect motor neurons in cell culture [97].

Two studies by Falk et al. showed how VEGF-B serves as an inducible neuroprotectant in a model of neurodegeneration. In the first [98], researchers used rotenone, a neurotoxin and commonly used pesticide that produced a well-characterized PD model, on rat midbrain neuronal cultures. In this study results demonstrated an upregulation of several genes post-rotenone treatment: notably, an increased transcriptional activation of VEGF-B and unchanged levels of VEGF-A. The same in vitro study using rat midbrain cultures, rotenone was used either alone, or with the addition of VEGF-B one hour prior to rotenone treatment. Results showed a significant loss of DAergic neurons compared to untreated control cells while, in cultures treated with VEGF-B, there was a neuroprotective rescue of cell loss. In the second [99]. VEGF-B’s neuroprotective effects were tested in vivo using a unilateral 6-OHDA rat PD model, with an intrastriatal injection of VEGF-B prior to the neurotoxic treatment. In VEGF-B-treated animals there was an improvement in PD behavior, partial protection of DAergic fibers in the striatum, and a partial rescue of DAergic neurons in the SN, indicating a neuroprotective effect.

Additionally, in two studies by Yasuhara et al., VEGF was found to be neuroprotective against cell death in mesencephalic neurons in vitro [100]. Further, in vivo using rat 6-OHDA models, VEGF was continuously infused into the striatum where it preserved DAergic neurons from insult and reduced the number of rotations in the amphetamine-induced rotational behavior test [101]. Results indicated that VEGF promotes neuroprotection indirectly by activating the proliferation of glia and promoting neurogenesis.

**IGF-1**

The insulin superfamily of peptides consists of insulin and insulin-like growth factors 1 and 2 (IGF-1, IGF-2) [102]. Here, we focus on IGF-1 because it is produced by all cells types in the mature brain and is diffusely expressed in many neuronal structures including the cerebellum, brainstem, and spinal cord [103]; while IGF-2 is produced at high levels during development, and is mostly restricted to the choroid plexus and meninges [104]. IGF-1 signals through the insulin-like growth factor 1 receptor (IGF-1R) and is regulated by IGF-binding proteins (IGFBPs).

IGF-1R is a receptor tyrosine kinase that, upon activation and autophosphorylation, signals through the PI3K/Akt and MAPK signaling cascades [105,106]. IGF-1 has been proven to exert neuroprotective and proliferative function via promotion of cell survival, prevention of apoptosis, and stimulation of neurogenesis [107]. It is believed that IGF-1 has a role in PD, rather than general survival of neurons in the brain, due to the following: (i) the SN is one of the regions in the brain that possesses a substantial density of IGF-1Rs [108], (ii) IGF-1 increases survival of neurons in the brainstem including the SN [109], and (iii) IGF-1 rescues DAergic neurons from programmed cell death [110].

It has been reported that IGF-1 has a positive effect on DAergic neurons in both in vitro and in vivo models. Using rat cerebellar neurons, Offen et al. [111] treated cell cultures with high concentrations of DA to induce oxidative stress and cause neurodegeneration with or without
administration of IGF-1. Addition of IGF-1 to DA-treated cells significantly increased cell survival and expression of antiapoptotic proteins [106]. Another in vitro study using neuronal cultures reported an IGF-1-mediated rescue of aggregated α-syn toxicity via the activation of PI3K/Akt [112]. Lastly, in an in vivo model, activation of the IGF-1R protected against DAergic neuron loss in the SN, and alleviated motor deficits in balance and coordination that were induced by 6-OHDA in rats [113].

MANF and CDNF

In addition to the other well-known NTFs, more recent investigation has displayed that MANF and CDNF possess neurotrophic effects on nigral DAergic neurons and neuroprotective effects in animal PD models. Importantly, high levels of MANF and CDNF are detected in many brain regions including the striatum and SN [114] and protect against DAergic cell death in 6-OHDA and MPTP models [114]. In vivo, prior to a 6-OHDA lesion, CDNF was administered, and induced protection of DAergic neurons in the SN. CDNF was as efficient as GDNF in producing long-lasting beneficial motor effects and reduced amphetamine-induced asymmetrical turning behavior [115,116].

MANF and CDNF appear to be important for ER homeostasis as they are largely confined to the endoplasmic reticulum (ER), by a KDEL sequence [115,117]. The belief that these NTFs are of high importance to the ER was further backed up with the finding MANF can bind to KDEL receptors and ER molecular chaperones [117], which will be discussed in further detail later. These NTFs can also be secreted to exert their neuroprotective effects in times of excessive ER stress [118]. Many modes of action of these NTFs have been postulated. One possible mechanism is the activation of intracellular signaling pathways PI3K/Akt and PLCγ [114].

Others NTFs

There are several other NTFs that have been identified which possess similar functions to those previously mentioned. NGF was the first NTF to be discovered and is the canonical member of the NGF-superfamily, composed of previously discussed BDNF and neurotrophins (NT)-3, -4/5, and -6 [119]. Aside from BDNF, the NGF-superfamily is less implicated than other NTFs in PD, as it mainly functions on sympathetic and sensory neurons in the peripheral nervous system (PNS) and in the development and maintenance of cholinergic neurons in the basal forebrain [120]. However, NGF and NT-3, through binding to their receptors, TrkA and TrkC, respectively, are known to activate several intracellular pathways including MAPK, PI3K/Akt, and PLCγ [121] to promote differentiation and survival of neurons. Like BDNF, the remaining NGF-superfamily members are also synthesized as proNTFs, which show preferential binding for p75NTR [122].

CNTF, belonging to the neurotrophic cytokine family, which also includes leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), and interleukin 6 and 10 (IL-6,10), supports the survival of motor and DAergic neurons [123,124]. CNTF signals through a tripartite complex of CNTFRα, LIFRβ, and GP130, activating several downstream signaling cascades such as MAPK and PI3K/Akt. Activation of these pathways result in increased transcription of several NTFs,
including FGF and IGF-1 [125,126]. CNTF has also shown an ability to mediate DAergic innervation and promote neuroplasticity and neurogenesis of DAergic neurons from the subventricular zone (SVZ) and dentate gyrus in adult mice [127,128].

The fibroblast growth factors (FGFs) are a large family of 22 members with diverse functions in development, metabolism, and neural activities [129]. The FGFs bind to the fibroblast growth factor receptor (FGFR), a receptor tyrosine kinase, which autophosphorylates and activates several downstream pathways including MAPK, PI3K/Akt, and PLCγ [130,131]. Several FGFs have been implicated in PD. For example, FGF20 binds to a receptor (FGFR-1C) that is highly expressed in the SN and, through activation of the MAPK pathway, is essential for the survival of DAergic neurons [132]. Similarly, FGF2 has been shown to have neuroprotective effects on DAergic neurons both in cultured neurons and in a rat 6-OHDA PD model [133]. Lastly, basic fibroblast growth factor (bFGF) has shown to be neuroprotective in 6-OHDA lesioned rats and in culture by activation of the PI3K/Akt pathway to promote survival [131].

**Neurotrophic Factor Effects on Transcription Factors**

NTF signaling stimulates many signaling cascades, in this review, we concentrate on the aforementioned MAPK, PI3K/Akt, and PLCγ pathways (Fig 4). These pathways activate numerous transcription factors, such as cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), triggering expression of genes involved in cell survival and maintenance of cellular function, while inhibiting pathways leading to cell death [121,134,135,136]. The exact mechanisms and specific examples will be discussed in appropriate sections. Paramount to the survival of DAergic neurons, CREB is directly involved in regulating TH expression in PD [137,138], where tyrosine kinase receptor activation is responsible for CREB transcription, thus, playing a crucial role in exercise-dependent neuroplasticity [70]. Furthermore, PA induces neuroplasticity, not only under normal physiologic conditions, but also in times of CNS insult. This PA-induced neuroplasticity occurs by increasing TH enzymatic activity, acting on the survival and synthesis of DAergic neurons [139].

![Figure 4. NTF action on cells.](image-url)

Schematic displaying NTFs general mechanism on cells leading to activation of intracellular signaling cascades and signal transduction. Action on transcription factors leads to cell survival, maintenance of function, growth, and synaptic plasticity. TH is responsible for synthesis of DA and is under control of the transcription factor CREB. Upon activation, CREB increases TH activity.
NEUROPLASTICITY AND SYNAPTIC TRANSMISSION

Neuroplasticity refers to the ability of the nervous system to respond and adapt to environmental challenges and encompasses structural and functional mechanisms that may lead to neuronal remodeling, formation of novel synapses, and neurogenesis [140]. Aside from their known function in supporting neuronal survival, it is now well established that NTFs mediate neuroplasticity in adulthood where they modulate axonal and dendritic growth and remodeling, membrane receptor trafficking, neurotransmitter release, and synapse formation and function [141].

Neurogenesis occurs when neural progenitor cells (NPCs) undergo asymmetric divisions and can produce neurons or glial cells. Numerous signaling pathways are involved in determining the fate of NPCs including growth factors, cytokines, and neurotransmitters [142]. Glutamate is a neurotransmitter that indirectly stimulates neurogenesis via the production of NTFs, namely BDNF and IGF-1, which promote neurogenesis [143,144]. Furthermore, BDNF’s neurogenic action is potentiated through a positive feedback loop with nitric oxide (NO) in differentiating neurons, to inhibit NPC proliferation and promote neuronal differentiation [145]. In a study by Tajiri et al [50]. 6-OHDA lesioned rats were subject to treadmill exercise to investigate motor function, cell survival and changes, and NTF levels. Results showed motor function improvement, preservation of TH-positive fibers and DAergic neurons in the striatum and SN, and increased levels of BDNF and GDNF. Importantly, using neuronal staining, significantly higher numbers of cells were located at distances of 100-200µm and 200-400µm away from the SVZ.

DA deficiency in PD leads to structural changes in synapses and functional changes in the form of neurotransmitter imbalance. On the structural and functional levels, the most well-known interactions in the basal ganglia (called the striatum in rodents) are on dendritic spines of medium spiny neurons (MSNs), which serves as the interface for signaling of the DAergic and glutamatergic systems [146,147]. Dendritic spine loss of MSNs has been observed in animal models of PD with DA depletion [148]. The neurotransmitter imbalance brought on by DA loss can lead to excessive activation of glutamate receptors, called excitotoxicity. As the name implies, excitotoxicity can kill neurons, especially those under conditions of reduced energy supply or increased oxidative stress during neurodegeneration [149]. These changes correspond to increased glutamatergic signaling and hyperexcitability of neurons [147]. The prolonged and elevated levels of glutamate within the synapse results in longer depolarization and ion homeostasis disturbances, ultimately leading to cell death.

One possible neuroprotective effect of PA may be restoration of structural basal ganglia circuitry through increases in spine density (Fig 5). In MPTP-lesioned mice, Toy et al [55]. found that a 90% loss in DA led to a ~20% dendritic spine loss in MSNs. However, with 6 weeks of treadmill exercise after the lesion, results showed reversal of dendritic spine loss, enhanced dendritic arborization, and increased synapse formation shown by expression of both presynaptic (synaptophysin) and postsynaptic (PSD-95) proteins. These same results were demonstrated in a second study by Shin et al [47]., however, they also discovered an amelioration in DAergic neuron loss. Both experiments also presented that exercise normalized MPTP-induced motor deficits.
Evidence shows that NTFs are responsible for the outgrowth of axons and dendrites [70]. This can be illustrated by two experiments [150,151] that show a knockout of BDNF or its receptor, TrkB, led to dendrite and spine reduction in several brain regions, with MSNs being especially sensitive. Furthermore, in mesencephalic DAergic neurons, NTF increases the length and branch number of neurites on DAergic cells [152]. The growth-promoting effect on neurites is mediated by both transcription-independent and -dependent mechanisms, meaning NTFs may influence local cytoskeletal dynamics via Ca2+ and kinase-mediated mechanisms, or induce expression of genes that encode cytoskeletal proteins and cell adhesion molecules [142]. For example, the MAPK pathway, which is activated by several NTFs, is involved in synapse formation and plasticity, and acts at both the cytoplasmic and nuclear level [153].

NTFs have also been shown to regulate synaptic transmission and neurotransmitter concentration by enhancing ion channel function [155], increasing neurotransmitter levels [156], and increasing Ca2+-binding proteins involved in neurotransmission [157]. For example, bFGF, NGF, and BDNF have shown to protect against glutamate toxicity by inducing antioxidant defense systems and stabilizing Ca2+ concentrations [158]. Additionally, through their action at the presynaptic terminal, NTFs influence synaptic vesicle docking, fusion, and recycling [159]. This is highlighted by the action of BDNF on the presynaptic proteins like Synapsin I. BDNF acts through PLCγ in this pathway to modulate Synapsin functions, including increased neurotransmitter release, transporter regulation, and regulation of vesicular release [70].

Another possible neuroprotective effect of PA may be functional restoration of neurotransmission (Fig 6). With 4 weeks of exercise in PD rat models, Chen et al [154]. reported a normalization of the increased glutamate levels and decreased excitotoxicity to MSNs. Importantly, yet somewhat redundantly, this review has provided an abundance of evidence to show that exercise increases DA levels in the brain. This is highlighted in work by Tillerson et al [53], where they show that up to 4 weeks of treadmill exercise in a rat 6-OHDA model of PD
increased striatal levels of DA, DA metabolites (DOPAC, HVA), DA transporters (DAT, VMAT), and survival of DAergic cells. These changes are likely due to the action of NTFs, shown by their ability to increase supply of transcription factors that induce genes responsible for DA metabolism, including TH and AADC [89]. Taken together, PA-dependent neuroprotection may work through a mechanism that mitigates corticostriatal hyperexcitability, modulates DA signaling, and/or diminishes glutamatergic neurotransmission [160].

**Figure 6. NTF-mediated functional restoration with PA.**
(A) With PA, NTF upregulation causes increases in DA, DA metabolites DOPAC and HVA, DA transporters DAT and VMAT2, and synthesis enzyme TH in Tillerson et al [53]. (B) A representative image of the DA synthesis, transport, and metabolism from Cai et al [161].

**ANGIOGENESIS AND VASODILATION**

PA induces changes including improved synaptic plasticity and neuroprotection that may be conferred by an increase in the number (angiogenesis) and diameter (vasodilation) of cerebral blood vessels (Fig 7a) following exercise in several brain regions including the motor cortex, cerebellum, and midbrain [162,163]. There is also evidence of a relationship between angiogenic factors and neurogenesis as neurogenic regions, like the SVZ and dentate gyrus, where new cells are clustered close to blood vessels and proliferate in response to vascular growth factors [164,165,166]. Vascular changes resulting from exercise are likely mediated by VEGF and IGF-1 (Fig 7b,c), as gene expression of these growth factors were increased with exercise and a blockade of peripheral VEGF and IGF-1 inhibited the neurogenic increase observed with exercise in rodent models [167,168].

In one study, cerebral angiogenesis was induced in aging rats that were exercised on a treadmill 30 min/day for 3 weeks. An increase in the density of microvessels was found within the cerebral vasculature, as well as an increase in angiogenic factors such as angiopoietin 1,
angiopoietin 2, and VEGF [169]. Another study demonstrated a similar result in an MPTP-induced PD mouse model that were treadmill-exercised 40 min/day for 4 weeks. Results showed increases in blood vessel density and striatal angiogenesis markers VEGF and CD34 in exercised PD mice compared to controls [32].

![Diagram of NTF action on blood vessels](image)

**Figure 7. NTF action on blood vessels.**
(A) Schematic displaying increases in the number and diameter of blood vessels due to NTF action. (B,C) Results from Falk et al. [99], displaying NTF action on neurons provides neuroprotection in addition to blood vessel changes.

Increased angiogenesis and vasodilation improve microcirculation around DA neurons and fibers in the brain. This likely promotes survival of DA neurons via several indirect mechanisms including: (i) increased availability of NTFs and biomolecules to neuronal cells through increased circulation and vascular permeability, allowing for survival and enhanced functionality; (ii) removal of waste materials and maintenance of cellular homeostasis; (iii) proliferation and targeted passage of glial cells, causing an upregulation in antioxidant and NTF secretion [100,147,170].

**OXIDATIVE STRESS AND MITOCHONDRIAL FUNCTION**

Mitochondria are essential for many diverse processes in the cell, including energy metabolism, calcium buffering, and apoptosis. These organelles are often referred to as the “powerhouse of the cell” because they generate the cellular supply of energy in the form of ATP. They are also the main source of reactive oxygen species (ROS) which, at normal physiological levels, play important roles in several cellular signaling pathways. Therefore, they are most susceptible to damage by oxidative stress on DNA, lipids, and proteins [171]. Accumulations of ROS can lead to mutations in mitochondrial DNA and misfolding/aggregation of proteins, which can disrupt normal cellular functions [172]. Protein misfolding and aggregation will be discussed in the next section on protein misfolding and mitochondrial function. Neuronal cells are especially vulnerable to the functional deterioration of mitochondria, mostly due to their high energy demands, which has been highly implicated in PD [173,174].
It has been discovered that there are higher amounts of common deletions in mitochondrial DNA of the surviving DAergic neurons in the SN and are believed to be the result of oxidative damage [175]. The “mitochondrial dysfunction” hypothesis of PD was reinforced upon discovery of mutations in several genes (e.g., PINK1, DJ-1, PARK2, Parkin) that encode mitochondrial proteins, giving rise to an inherited form of PD [176,177]. Genetic models of PD also provide evidence for increased oxidative stress. Loss of PINK1 function was associated with increased ROS production in midbrain neurons by high Ca2+ ion concentrations in the cytoplasm, leading to superoxide overproduction. ROS production subsequently inhibits the membrane glucose transporter causing dysregulation of mitochondrial metabolism [178,179].

Elevated cell death in the SN due to oxidative stress may occur due to (i) greater ROS production because of increased DA turnover, (ii) an attenuated ability of the brain to rid of ROS because of antioxidant deficiency (e.g., glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)), or (iii) production of ROS brought about by elevated levels of neurochemical insult, such as heavy metals [180,181]. It has been suggested that depletion of GSH in astrocytes due to increased oxidant production could decrease glia mediated GSH release, and thus, deplete GSH in neurons [182] and increase exposure to ROS. Rotenone- and MPTP-induced PD animal models have both demonstrated the oxidative stress model. MPP+ and rotenone inhibit ATP synthesis by blocking electron transport along the electron transport chain (ETC), specifically, through inhibition of complex I, resulting in the overproduction of ROS. This provides evidence to the view that MPP+ is a mitochondrial toxin, and its neurotoxic effects can be effectively prevented by antioxidants [176,183,184].

The ability for NTFs to prevent cell death, through reduction in oxidative damage, has been shown in vitro. DAergic SHSY-5Y cells treated with 6-OHDA or MPP+ displayed cell death due to oxidative damage, shown by a 5-fold increase in oxidized GSH. Treatment of the cells with BDNF, however, prevented the rise in oxidized GSH and increased levels of the protective enzyme GSH reductase, preventing cell death [185]. A similar result of NTF-neuroprotection to oxidative damage was seen in mesencephalic cell cultures treated with 6-OHDA. bFGF treatment of cells increased levels of GSH, which was also noted in cells treated with 6-OHDA alone. This upregulation of antioxidant defense systems from bFGF was further validated by treatment with a GSH inhibitor, which diminished bFGF protection [186], and that neuronal cells upregulate transcription of GSH in times of oxidative stress from 6-OHDA or H2O2 [170].

Reduction in oxidative stress has also been noted in several in vivo studies that examine exercise-induced production of antioxidants. A study by Tuon et al [54]. reports that 8 weeks of treadmill training in rat 6-OHDA PD models increased striatal levels of antioxidant enzymes SOD, CAT, and GSH peroxidase, and reduced oxidative damage to lipids and proteins. These results coincided with increased levels of BDNF, TH, and a reduction in the apomorphine-induced rotational test. Several other studies report exercise-induced decreases in oxidative stress caused by NTF-mediated upregulation of antioxidant systems [42,158].

In a more recent study [187], 4 weeks of treadmill exercise in a rat MPP+ model of PD led to expression of nuclear factor erythroid 2-related factor 2 (Nrf2), a regulator of expression of
several antioxidant enzymes in the nigrostriatal system. Exercise-induced expression of Nrf2 prevented the MPP+-induced downregulation of antioxidant enzymes and protected nigrostriatal DAergic neurons from degeneration. It has previously been reported that Nrf2 resides in the cytoplasm and, only after suitable oxidant stimulation, translocates to the nucleus [250]. It has since been discovered that BDNF controls one avenue of Nrf2 translocation to the nucleus to upregulate production of antioxidant enzymes [189]. Other NTFs, MANF and CDNF, display an antioxidant-like protective role against oxidative stress from the ER [115], which will be discussed in the proceeding section on protein misfolding.

Although this review will not provide an in-depth look at mitochondrial mechanisms of apoptosis, it is important to understand that these organelles participate in cell death by releasing various cytotoxic proteins in response to numerous signals or lack thereof. These proteins, such as cytochrome c, are released from mitochondria through a membrane pore and activate caspases in the cytoplasm and nucleus, which dismantle cellular contents and signal for efficient phagocytosis of cellular corpses by microglia. Because of the lethality of this process, tight regulation is required to prevent accidental activation. Regulation of mitochondrial permeability is carried out by the pore-forming Bcl-2 family proteins, composed of two classes: antiapoptotic proteins, notably Bcl-2, and proapoptotic proteins, notably Bax. Further regulation occurs in the cytoplasm where inhibitors of apoptosis (IAP) proteins exert an inhibitory effect on caspase activity. To override inhibition of IAP action, proteins such as Smac (also called DIABLO) and Omi (also called HtrA2), cause apoptosis by binding to or degrading IAPs, respectively [190]. For a more detailed review of apoptosis, please reference the appropriate literature.

As previously discussed in the section molecular mechanisms of neurotrophic factor action, many NTFs trigger intracellular signaling pathways MAPK, PI3K/Akt, and PLCγ. Activation of these pathways results in upregulation of antiapoptotic proteins, including Bcl-2 and Bcl-X, and downregulation in proapoptotic proteins such as Bax, BAD, FOX, JNK, p38, and p53. Adjustments in apoptotic proteins by NTFs diminishes release of cytochrome c from the mitochondrial membrane and extinguishes caspase activation [121,134,135,136,191].

Exercise-induced protection of DAergic neurons has also been shown to occur via improvements in mitochondrial function. In a mouse model of PD, mice that exercised on a treadmill for 18 weeks beginning 1 week before 5-week treatment with MPTP exhibited improved mitochondrial function, displayed by increased oxygen consumption, oxidative respiration, and ATP production. Furthermore, through upregulation of BDNF and GDNF, results showed that improved mitochondrial function leading to DAergic neuron survival was NTF-selective and region specific, as NTFs were elevated predominantly in the SN [42].

A similar result was seen in Koo et al [40], where MPTP administration followed by treadmill training for 8 weeks reduced DAergic neuron loss by altering mitochondrial protein levels in three ways: (i) apoptotic pathways were shunted due to an increase in Bcl-2 and decreases in both Bax and caspases; (ii) expression of mitochondrial membrane transport proteins, responsible for import of structural and functional proteins, was elevated; and (iii) there was an increase in electron transport chain proteins, which ameliorated mitochondrial function.
Although the detailed mechanisms are exceedingly complex and not fully understood, NTFs likely participate in a positive feedback loop with increases in metabolism to reduce oxidative stress and improve mitochondrial function, rather than initiate the processes on their own. This can be exemplified by preceding discussion on exercise-induced PGC-1α production. It was shown that exercise increases levels of PGC-1α, leading to increased metabolism and production of metabolic intermediates, which then caused an upregulation of NTFs [61,62]. PGC-1α expression not only causes mitochondrial biogenesis, it also increases the functionality of mitochondria by increasing mitochondrial protein production, enzyme activity, and mitochondrial DNA [192]. Marked increases in metabolism also incur increased oxygen consumption, which would decrease production of ROS, and thus, oxidative damage on mitochondrial DNA leading to dysfunction and protein aggregation.

**PROTEIN MISFOLDING AND MITOCOCHONDRIAL FUNCTION**

The detection of Lewy bodies (LBs) in the SN and other brainstem nuclei has long been considered the pathogenic hallmark of PD. During the development of PD, cellular defense mechanisms to deal with misfolded protein aggregates, including the unfolded protein response (UPR), molecular chaperones, the ubiquitin-protease system (UPS), and autophagy are compromised [193]. LBs are protein aggregates of α-syn and other biomolecules from microtubules, mitochondria, lysosomal and autophagy pathways [194].

Studies show that mutant protein, α-synuclein (α-syn), can induce the UPR in the ER, which induces adaptive cell death in response to pathologic conditions such as nutrient deprivation,
disruption of Ca2+ homeostasis, viral infection, and secretory protein mutations [195]. In addition, α-syn in LBs also block ER vesicles from docking with the Golgi apparatus leading to vesicular accumulation [196]. MANF and CDNF are important in the reduction of ER stress and prevention of apoptosis through UPR [115]. These NTFs were shown to be upregulated with exercise and act as a neuroprotectant in a 6-OHDA rat PD model. Results of the study showed a decrease in the apomorphine rotation test, increased DA concentration, and ameliorated levels of DAergic neurons [43,197]. Furthermore, the lack of MANF in a mouse model leads to activation of UPR [198]. The exact mechanism of neuroprotection was, however, elucidated in cell studies.

In two studies using DAergic SHSY-5Y cells, 6-OHDA or α-syn overexpression were used to model ER stress-induced apoptosis. These studies found that MANF treatment significantly upregulated the molecular chaperones HSP70 [199] and GRP78 (also called BiP) [200]. In addition, they saw a marked decrease in caspase activation. This suggested that upregulation of molecular chaperones, involved in the rescue of misfolded proteins [201], alleviates ER stress and protects DAergic neurons from apoptosis. Both studies validated their predicted mechanisms by overexpressing the molecular chaperone of interest to inhibit 6-OHDA-induced apoptosis, and by using knockouts of the molecular chaperone to block MANF-induced cell survival.

These NTFs can also be secreted to exert their neuroprotective effects in times of excessive ER stress. For example, MANF can bind to and block the proapoptotic molecule Bax, preventing cell death [118]. Furthermore, they have also demonstrated binding and internalization of oxidized lipids at the cell surface, reducing ER stress and UPR pathways [114].

The UPS is the major non-lysosomal pathway for protein breakdown (proteolysis). The destination of the UPS, the proteasome, is a complex of enzymes responsible for intracellular proteolysis of proteins marked for degradation by ubiquitin tags. Alterations in proteasome function of protein clearance have been implicated in PD, where formation of abnormal protein aggregates (e.g., LBs), resulting from proteasome inhibition, activate intracellular UPRs, causing oxidative stress and apoptosis [202]. It still remains unknown if NTFs act directly on the UPS to promote protein aggregate clearance and cell survival, or if NTFs simply ameliorate the survival of neurons despite dysfunction and apoptotic signals; however, several theories have been proposed. Two of which being that (i) NTFs stimulate UPS-mediated proteolysis through enhancing the expression of ubiquitin enzymes and proteasome subunits, and (ii) NTFs preserve proteasome function and attenuate toxicity of proteasome inhibition [203].

The first hypothesis was shown in Jho et al [204], where CNTF upregulated the 20S proteasome, a component of the proteolytic core. However, this result was obtained from hepatic cells and it is unknown if CNTF would have the same effect on neurons. The second hypothesis was shown using BDNF and GDNF. Santos et al [205], suggested that BDNF downregulated proteasome activity in cultured hippocampal neurons and Du et al [206], found GDNF protects nigral DAergic neurons in a PD mouse model from UPS impairment-induced degeneration.

Lastly, autophagy is an autonomous cell survival mechanism that maintains homeostasis by catabolically degrading misfolded proteins and clearing excess or damaged organelles by isolating them in double-membraned structures, eventually fused with lysosomes [207]. BDNF is
known to signal in several pathways. One of which, the PI3K/Akt pathway, is upstream of the mammalian target of rapamycin (mTOR), which is fundamental in the induction of autophagy [208]. While mTOR is most often discussed for its role in autophagy, it is also involved in several cellular processes, including protein synthesis, cell growth, proliferation, survival, and synaptic plasticity [209].

In one study, researchers investigated the neuroprotective effect of BDNF through the PI3K/Akt/mTOR pathway in cortical rat neurons, subject to oxygen deprivation (hypoxia). Their results demonstrated that BDNF acted as a neuroprotectant by promoting cell viability via the upregulation of autophagy, which is essential to maintaining cellular homeostasis when responding to stress [208]. On the other side of the mTOR pathway, CNTF was shown to activate a signaling molecule, Rheb, leading to protection of rat SN DAergic neurons [128].

![Figure 9. Cellular protein clearance pathways.](Penke et al [210])

Components of several intracellular protein clearance pathways are upregulated through NTF action including molecular chaperones, the ubiquitin-proteasome system, and autophagy. Derived from Penke et al [210].

Similar to the discussion in the previous section, decreases in protein misfolding/aggregation cannot be entirely attributed to NTF action, but rather, NTF action in combination with improved mitochondrial function. Through increased oxygen consumption and decreased oxidative damage to mitochondrial DNA and proteins, there would be a reduced amount of misfolded proteins and an increase in antioxidant enzymes which would reduce oxidative damage [211]. The previous section discussed how oxidative damage to mitochondrial DNA leads
to mutations in inherited forms of PD. Here, the aberrant functionality of these gene products can be demonstrated using two of the most common inherited gene mutations, Parkin and PINK1. Narendra et al [212]. showed that these two gene products are involved in a mitochondrial quality control pathway that identify dysfunctional mitochondria and mark them for destruction by autophagy. Similarly, Moscovitz et al [213]. found that the DJ-1 gene plays an important regulatory role in the function of the 20S proteasome – part of the UPS.

**NEUROINFLAMMATION**

PA’s neuroprotective effect has been linked to the modification and prevention of the neuroinflammatory process [214], which has been shown to play a role in the pathogenesis of animal PD models via the increase of pro-inflammatory cytokines, chemokines, and ROS that trigger the initiation of extrinsic neuronal apoptosis [215] and activate both microglia and astrocytes in response to α-syn aggregates and necrotic neurons [216,217]. Glial cells may be activated in two different ways – a pro-inflammatory (classical M1 activation) or an anti-inflammatory (alternative M2 activation) response. In the M2-type response, microglia increase their expression of anti-inflammatory cytokines and NTFs including IL-6, IL-10, TGFβ, IGF-1, NGF, and BDNF [218]. Astrocytes also secrete anti-inflammatory compounds and NTFs such as GDNF, BDNF, and MANF, serving to restore functionality and promote survival to injured DAergic neurons [219].

![Figure 10. NTF action on neuroinflammation.](image)

Schematic displaying M1 and M2 type responses to neurotoxic insults. NTF action promotes an M2 type anti-inflammatory activation, promoting cell survival and inhibiting pro-inflammatory activation leading to cell death.

In a study by Real et al [45]., 6-OHDA-lesioned rats were subject to treadmill exercise for 4 weeks or a sedentary lifestyle. Lesioned PDS animals displayed an increase in neuroinflammatory markers in the SN and striatum, as well as an increase in the astrocyte,
microglial, and oxidative species activation. On the other hand, the lesioned PDE animals did not show neuroinflammatory responses and performed better on the apomorphine-induced rotation test. Jang et al [39]. investigated the effects of exercise endurance in an MPTP PD mouse model, specifically monitoring the interplay between α-syn levels accompanied by neuroinflammation and the anti-inflammatory effects of exercise. Results showed increased motor function and reduced cell death by reestablishment of TH levels when compared to PDS controls. PDE mice showed a reduction in the α-syn protein and pro-inflammatory cytokines, preventing activation of apoptotic signaling cascades incurred by MPTP striatal administration.

One of the long-standing theories proposes that PA regulates immune function through enhanced secretion of muscle-derived cytokines and peptide hormones (myokines), including several known NTFs. Importantly, there have been shown to cross the BBB and act in an anti-inflammatory mechanism on the CNS by upregulating the expression of proteins with strong anti-inflammatory, anti-oxidative, and anti-apoptotic properties (e.g., metallothionein) [220,221]. Additionally, macrophages and T-regulatory cells produce NTFs, in response to secretions from skeletal muscles, which act in an anti-inflammatory manner to inhibit the production of pro-inflammatory cytokines (e.g., IL-1α, IL-1β, and TNF-α) [222].

The role of exercise induced NTF neuroprotection against inflammatory insult was perhaps best demonstrated in Wu et al [57]. by showing the BDNF signaling pathway protects DAergic neurons. Researchers allowed 4 weeks of treadmill exercise prior to injection of LPS to induce PD in a mouse model, brought on through microglia activation in the SN. Exercise prior to LPS treatment completely prevented the reduction in DAergic neurons, DA in the striatum, BDNF levels in the SN, and impaired motor coordination. In this study, results were attributed to the restored levels of BDNF signaling, protecting DAergic neurons against inflammation-induced insult.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Pharmacological and surgical treatment of PD are often ineffective, and the benefits seen decrease with disease progression, likely because the disease is multifactorial both in its cause and development. PA is non-invasive, -pharmacological, and causes no side effects. Although PA has many known benefits through the increased mobilization of NTFs, the mechanisms are not fully understood.

Studies in animal models of PD (Table 2) indicate that PA may prevent the loss of, protect, or restore DAergic neurons and improve motor function. PA likely enhances the overall metabolic state, which leads to an increased synthesis and release of NTFs. These NTFs then activate several signaling cascades to exert their effects on neuronal cell populations.

Studies in animal models elucidate how NTFs can improve functionality in many aberrant cellular mechanisms seen in PD, which this review has laid out in four main parts: (i) the brain is better equipped to adapt to abnormal structural and functional changes through neurogenesis, neuroplasticity, and refined neurotransmission; (ii) cerebral blood flow is improved through angiogenesis and vasodilation; (iii) mitochondrial function, and thus, metabolism are boosted by...
reducing oxidative stress and preventing protein misfolding/aggregation; and (iv) self-harm is eliminated by mitigating neuroinflammation.

Although, utilizing neurotoxins in animal models do not completely reproduce the etiology of PD in humans. While commonly used to study the pathogenesis, progression, and effectiveness of disease treatment, when a neurotoxin is administered, PD is induced, rather than being brought on systemically. Additionally, motor control in animal models is not as hierarchical and human locomotion receives more contribution from the cerebral cortex and subcortical structures.

The cellular mechanisms in this review on how PA mediates neuroprotection could be entirely practical; however, more research is needed to establish these mechanisms and elaborate their details. For translational and clinical research in humans, it is important to determine when to start and what form, intensity, and duration of PA is needed to produce the maximal behavioral, anatomical, and neurochemical benefits as a disease-modifying factor.


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