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To dopamine and beyond, a review of the mechanisms of Parkinson's disease

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Thesis

**TO DOPAMINE AND BEYOND, A REVIEW OF THE MECHANISMS OF
PARKINSON'S DISEASE**

by

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ABSTRACT

Parkinson's Disease is a disorder of the midbrain dopaminergic system with characteristic neurodegenerative patterns, recognized for its motor symptoms. The neurodegeneration is most prevalent in the substantia nigra pars compacta, while dopaminergic neurons in neighboring structures are comparatively spared. There are many possible explanations for this disparity, including differences in tolerance to oxidative stress, and vulnerability to α -synuclein aggregates. The substantia nigra is part of the basal ganglia, a network of nuclei in the midbrain and base of the forebrain which are responsible for coordinating voluntary movement. Dopamine has an inhibitory effect in the basal ganglia. It dampens signals to remove noise, so the basal ganglia circuitry is not hyperactive. In the absence of dopamine, the flow of information through the basal ganglia is disrupted. This results in tremor, bradykinesia, and rigidity, known as the classic triad. No cure currently exists and therapies are unable to slow disease progression, so treatments are aimed at symptom management. Degenerative processes in Parkinson's Disease occur rapidly, early in the disease progression, with about 60% neuronal death in the substantia nigra prior to diagnosis. There is a need for biomarkers or other signs which can be used to clinically to diagnose the disease at an earlier stage. In conclusion this paper provides suggestions for future lines of research.

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LIST OF ABBREVIATIONS

AAAH.....	Aromatic amino acid hydroxylase
AADC.....	Aromatic L-amino acid decarboxylase
ALDH.....	Aldehyde dehydrogenase
BG.....	Basal ganglia
COMT.....	Catechol O-methyltransferase
CSF.....	Cerebrospinal fluid
DAT.....	Dopamine transporter
DNH.....	Dorsolateral nigral hyperintensity
DOPAL.....	3,4-dihydroxyphenylacetaldehyde
EEG.....	Electroencephalogram
GABA.....	Gamma-amino butyric acid
GPCR.....	G-protein coupled receptor
Gpe.....	Globus pallidus externus
Gpi.....	Globus pallidus internus
HVA.....	Homovanillic acid
L-DOPA.....	L-3,4-dihydroxyphenylalanine
MAO.....	Monoamine oxidase
MPTP.....	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI.....	Magnetic resonance imaging
MSN.....	Medium spiny neuron
NET.....	Norepinephrine transporter

PD	Parkinson's Disease
PNMT	Phenylethanolamine N-methyltransferase
ROS.....	Reactive oxygen species
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr.....	Substantia nigra pars reticulata
STN	Subthalamic nucleus
TAN	Tonically active neuron
VTA	Ventral tegmental area

INTRODUCTION

Parkinson's Disease in its modern conception has a history dating back over 200 years. Dr. James Parkinson first formally described the condition in 1817, attributing the name 'shaking palsy', or *paralysis agitans*, to the set of signs and symptoms to which we have now affixed the Physician's eponym (Parkinson, 2002). In his initial paper, Dr. Parkinson referred to the palsy as "involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured" (Parkinson, 2002, p. 223). Indeed, the description by Dr. Parkinson alludes to the now-standard set of symptoms which are used clinically to diagnose Parkinson's Disease (PD). That set, often referred to as the 'classic' triad, is composed of baseline tremor, rigidity, and bradykinesia (DeMaagd & Philip, 2015).

The tremor is present in 70-90% of patients and is generally around 4-6Hz when resting. The rigidity in PD is present in 80-90% of patients, and is often described as 'cogwheel' rigidity. This pattern resists passive movement for a short time until tension is released and the motion clicks into the next position, where tension builds again as if it were guided by a halting cogwheel. Bradykinesia, also present in 80-90% of patients, is notable for slowness of movement and in some cases movement ceases entirely, a phenomenon known as akinesia. A fourth notable symptom of the disease is postural instability, which is not considered part of the classic triad but still has diagnostic relevance. Collectively these motor abnormalities are referred to as parkinsonism

(DeMaagd & Philip, 2015). PD, particularly in its later stages, is also associated with cognitive symptoms including dementia, delayed reaction times, and difficulty coordinating motor sequences (Breen & Lang, 2017; Helie et al., 2013).

In most basic terms, PD is a disorder of the extrapyramidal system, in that it involves tracts of the motor cortex that are not a component of the medullary pyramids (DeMaagd & Philip, 2015). It involves a wide range of neural structures in the midbrain and forebrain, and it has been shown that dopamine is a neurotransmitter playing a major role in the modulation of neurons in the basal ganglia. The connection between dopamine and disease progression in PD dates back to the mid-twentieth century, however, the exact role of dopamine and the pathophysiology of PD is still debated (Vogt Weisenhorn et al., 2016). Several researchers have found that parkinsonism motor symptoms present clinically once about 60% of the dopaminergic neurons in the substantia nigra have degenerated (DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016). Given the magnitude of this loss before clinically significant signs of disease appear, there is an obvious need to discover new methods for early detection (Breen & Lang, 2017; DeMaagd & Philip, 2015).

There are not yet any tests to directly confirm Parkinson's Disease, so a diagnosis is made clinically by confirming presence of parkinsonism features and then ruling out any other disease which has a similar presentation. Care must be taken to examine for other similar diseases and comorbidities, as many of these conditions have vastly different pathophysiology and would not respond to treatment geared towards Parkinson's Disease (DeMaagd & Philip, 2015). There are several cases where

parkinsonism is caused by conditions with a more favorable prognosis, such as drug-induced parkinsonism, and these can easily be treated by changing medications. This avoids burdening the patient with undue medical and social concerns. Other diseases which commonly present with parkinsonism include Alzheimer's disease, benign essential tremor, cerebrovascular disease, Lewy body dementia, Creutzfeldt-Jakob disease, and subdural hematoma, although this list is far from exhaustive (DeMaagd & Philip, 2015).

At present there is no cure for PD. Current treatments all are aimed at managing symptoms rather than slowing progression of degeneration (Mostile et al., 2017). Treatments involving dopamine replacement has been shown to effectively control the motor symptoms of the disease (Mostile et al., 2017), but these therapies do not stop underlying disease progression (Willis et al., 2012).

There are many reasons why we do not have a cure for Parkinson's disease. Little is understood about the molecular nature of PD. Its genetic origins, the possibility of detectable biomarkers, and even the mechanism underlying dopaminergic degeneration remain uncertain (DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016; Willis et al., 2012).

Neuroanatomy

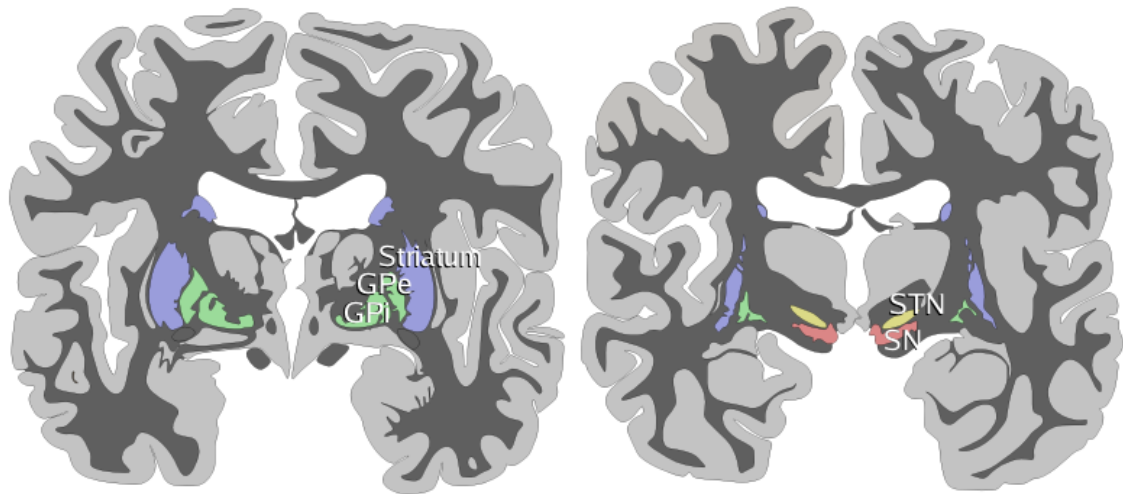


Figure 1 Coronal sections of Basal Ganglia. Source: Wikimedia Commons
<https://commons.wikimedia.org/wiki/File:Basal-ganglia-coronal-sections-large.png>

PD's etiology is rooted in the degeneration of dopaminergic neurons in the SNc, so this is a good structure with which to begin an explanation of the relevant neuroanatomy and neurophysiology. The SN is a BG structure located in the ventral mesencephalon. First described by Felix Vicq d'Azyr in 1784, the SNc is visibly darker than the surrounding material when observed in autopsy, thus earning its Latin name which translates to 'black substance' (Tubbs et al., 2011). The dark color results from high concentrations of neuromelanin, which is related to the dopamine synthesis pathway. Increased levels of neuromelanin indicate an increased presence of dopamine, and likewise low neuromelanin indicates low dopamine levels (Rabey & Hefti, 1990). There is evidence to suggest that neuromelanin concentration naturally increases with age (Tribl et al., 2009; Gibb & Lees, 1991), but in PD patients this neuromelanin is depleted

to the point that its absence is noticeable to the naked eye (Vogt Weisenhorn et al., 2016). About 60% of dopaminergic neurons in the SNc are depleted by the time parkinsonian motor signs are first clinically reported (DeMaagd & Philip, 2015).

There is a widely-accepted categorization method used to describe cell populations in the brain outlined by Dahlström and Fuxe (1964), which differentiated groups of brainstem cells from the medulla to the thalamus per their catecholaminergic behavior. In total there were 17 cell groups labeled A1-A17, of which nine groups are dopaminergic. The other groups are adrenergic or noradrenergic. A8-A16 are the dopaminergic groups, with A8-A10 in the ventral mesencephalon. A-9 is roughly aligned with the SN, mostly in SNc but some lesser presence in SNr, and A-10 matches up well with the ventral tegmental area (Dahlstroem & Fuxe, 1964).

Collectively, the A8-A10 cell groups, the anatomical structures they map onto, and the other non-dopaminergic neurons in the region are often referred to as the ventral mesencephalic dopaminergic complex (Yetnikoff et al., 2014). This area has 20,000-30,000 neurons in rodents, 160,000-320,000 neurons in monkeys, and 400,000-600,000 neurons in human brains. Despite the increased complexity of the human ventral mesencephalon compared to rodents, animal models have been shown to effectively account for the complex disease progression of PD (Vogt Weisenhorn et al., 2016). Much of the complexity in primates is in the SN; rodent dopaminergic neurons are found in equal number in the SN and VTA, but in humans the SN dopaminergic neurons far exceed those in the VTA (Brichta & Greengard, 2014).

The SN is divided into two functionally distinct subsections, the SN *pars compacta* (SNc) and the SN *pars reticulata* (SNr). The SNc is the primary locus of the dopaminergic neurons in the SN, and is the site most affected by neurodegeneration in PD pathology. Within the SNc, the most

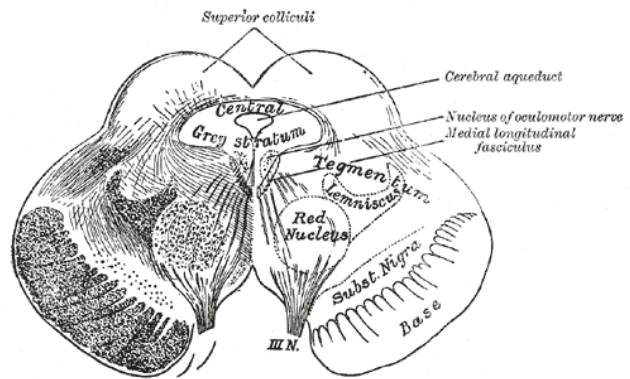


Figure 2 Location of substantia nigra in midbrain, as depicted in Gray's Anatomy (1918). Source: Wikimedia Commons, <https://commons.wikimedia.org/wiki/File:Gray712.png>

severe neurodegeneration is found in the ventrolateral aspect, particularly in extensions of ventral SNc dopaminergic neurons into the SNr, called nigrosomes. The SNr is relatively sparse in cells, and is functionally equivalent to the Gpi (Gibb & Lees, 1991; Vogt Weisenhorn et al., 2016). There is a third zone distinguished by cytoarchitectonic demarcations, the *pars lateralis*, located laterally and rostral in the SN, although this area has drawn relatively little attention in the PD literature (DeMaagd & Philip, 2015; Gibb & Lees, 1991).

The ventral tegmental area (VTA) is located medially to the SN and is just lateral to the midline of the brain. Similar to the SN, it has been implicated in a wide range of functions including addiction, reward, cognition, and addiction, and is closely connected to the limbic system (Johnson & North, 1992). Although the VTA and SN are somewhat similar from a macroscopic anatomical perspective, in the pattern of neurodegeneration observed in PD, the VTA, i.e. cell group A10, is largely unaffected, while cell group A9 in the SN is significantly diminished (Vogt Weisenhorn et al., 2016). The cell group A-8

is localized to the retrorubral field, an area of the ventral mesencephalon dorsolateral to the SN and caudal to the red nucleus. This area is notable for its interaction with the adrenergic system modulating dopamine release in the midbrain (Mejias-Aponte, 2016).

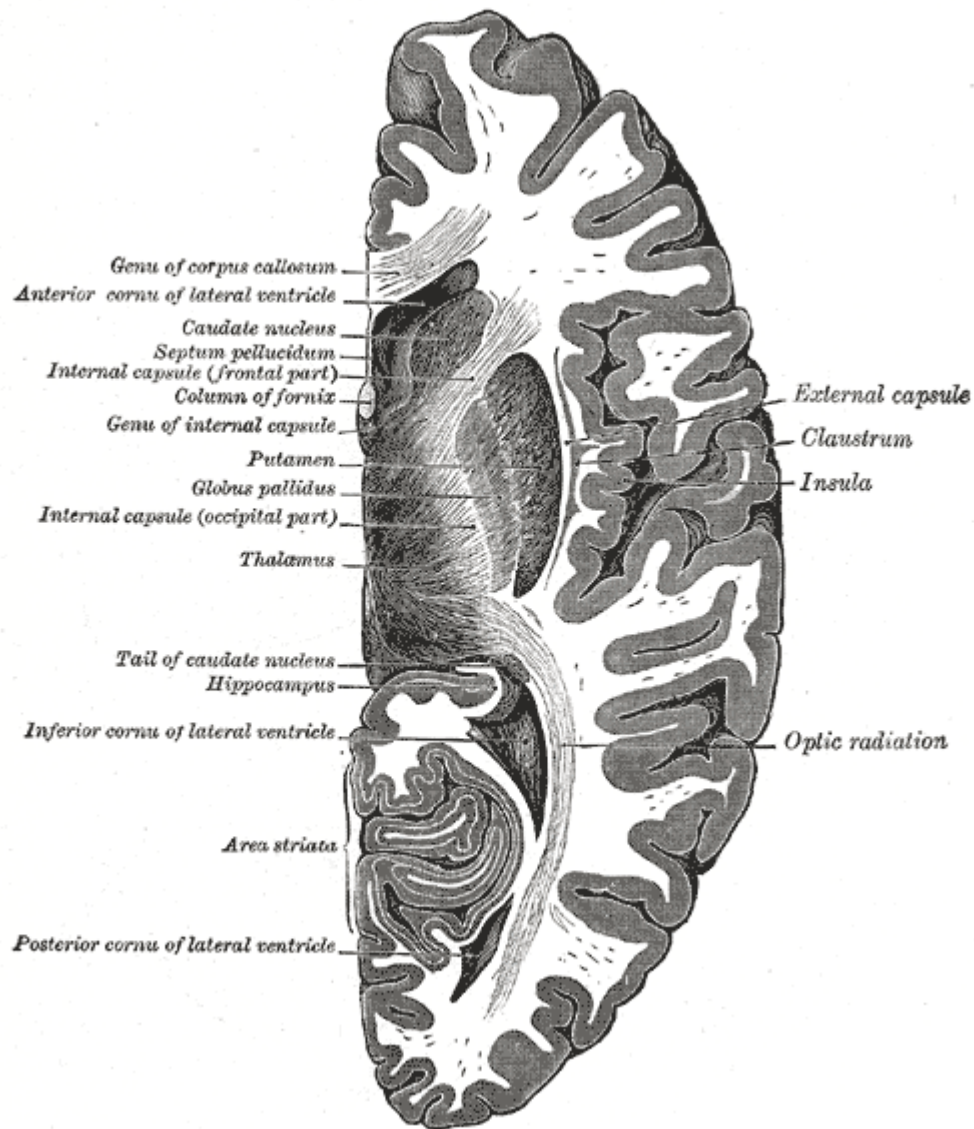


Figure 3 Organization of neuroanatomical structures, as depicted in Gray's Anatomy (1918). Source: Wikimedia Commons <https://commons.wikimedia.org/wiki/File:Telencephalon-Horiconatal.jpg#/media/File:Gray742.png>

Other nuclei in the BG include the striatum, the pallidum, and the subthalamic nucleus (Helie et al., 2013; Stocco et al., 2010). The striatum is a large structure at the

base of the forebrain. It is composed of dorsal and ventral subdivision. The dorsal striatum is further divided into a caudate nucleus and putamen, while the ventral striatum includes the nucleus accumbens and the olfactory tubercle. The pallidum is composed of the Gpe and Gpi (DeMaagd & Philip, 2015; Stocco et al., 2010). Considered in terms of neural computational processing, the striatum is the input to the BG because it is the recipient of major inputs from the entire cerebral cortex. The BG's outputs are the SNr and Gpi, which project to the thalamus (Stocco et al., 2010).

The striatum is the largest structure of the BG and is situated most laterally. Similar to the SN, it has been implicated in a wide range of cognitive and motor functions. The dorsal striatum is involved primarily with motor functions, reward systems, and neural inhibition (Stocco et al., 2010). A large white matter tract, the internal capsule, separates the two parts of the dorsal striatum. The striatum receives input from the cortex and projects it to other targets in the BG, most notable the SN. There is also a substantial amount of dopaminergic neurons from the ventral mesencephalon which converges upon the striatum, representing the major source of dopamine found within the striatum (Stocco et al., 2010; Vogt Weisenhorn et al., 2016; Yager et al., 2015).

Located caudally within the dorsal striatum, the caudate nucleus is implicated in motor processes and has been shown to be involved in non-motor processes as well, such as rewards and learning systems (Helie et al., 2013; Stocco et al., 2010; Weinberger & Dostrovsky, 2011). The caudate nucleus is involved in the pathways that give rise to a wide range of neurological diseases, including PD, Huntington's Disease, attention

deficit hyperactivity disorder, Alzheimer's Disease, obsessive compulsive disorder, schizophrenia, and more (DeMaagd & Philip, 2015; Mejias-Aponte, 2016; Stocco et al., 2010; Vogt Weisenhorn et al., 2016). The putamen, located rostral to the internal capsule, regulates movement and influences learning (Helie et al., 2013; Stocco et al., 2010). Occasionally the putamen is grouped together with the globus pallidus, and referred to as the lentiform nucleus. The list of processes and conditions which involve the putamen are just as extensive as the caudate nucleus (Stocco et al., 2010).

The ventral striatum, composed of the nucleus accumbens and the olfactory tubercle, is located lateral to the globus pallidus, and ventral to the dorsal striatum as the name suggests. It is intricately connected to the limbic system, and likewise plays a crucial role in reward systems and decision making behavior (Stocco et al., 2010). The nucleus accumbens contains predominantly GABAergic neurons, which are modulated by dopaminergic projections primarily from the VTA (Yager et al., 2015). It is indirectly associated with the degeneration of PD, but plays a direct role in rewards learning and addiction (Mejias-Aponte, 2016; Yager et al., 2015). The olfactory tubercle is responsible for transmitting sensory data relating to olfaction to relevant areas of the brain, but there is inconclusive research to show whether the ventral striatum participates in olfaction or not (Yager et al., 2015). Interestingly, anosmia is a symptom of PD so there may yet be a connection (DeMaagd & Philip, 2015).

Located medial to the striatum lies the globus pallidus. This structure is part of the telencephalon, and is has close functional ties to the subthalamic nucleus and several of the BG nuclei. Latin for 'pale globe', its name is possibly a reference to the high

proportion of myelination noted in the region, although the ultimate origin of the name is uncertain (Vogt Weisenhorn et al., 2016). The globus pallidus is also divided into two sub-regions by a sheet of tissue known as the medial medullary lamina, the globus pallidus internus (Gpi) and the globus pallidus externus (Gpe). Each nucleus is entirely encased in a myelinated covering. The Gpe is closest in proximity to the striatum, and the Gpi is situated medially and slightly ventrally to the Gpe (Helie et al., 2013; Weinberger & Dostrovsky, 2011). Collectively, the Gpe and Gpi work in conjunction with other BG nuclei and are involved in the production of voluntary movement. The Gpe and Gpi both receive input from the striatum, and the Gpi additionally receives input from the subthalamic nucleus. There is also a lesser degree of communication with the SN. The striatal afferents to the globus pallidus predominantly GABAergic, while the SN axons are dopaminergic. Both parts of the globus pallidus ultimately project to the thalamus (Helie et al., 2013). Despite being physically separated by a tract of white matter, the SNr and Gpi are alike in cytologic composition as well as function. This leads some researchers to consider the two as a single heterogeneous entity (Stocco et al., 2010).

There is a third pallidal component which is not often discussed in the PD literature concerning the midbrain dopaminergic system, the ventral pallidum. This is located in the substantia innominata, and relays information from the ventral striatum to the thalamus. The ventral pallidum contains a strong pleasure center, and is likely closely associated with the nucleus accumbens. This structure provides a link between the cortex and limbic system (Berridge & Kringelbach, 2015).

The final structure in the BG is the subthalamic nucleus (STN). This structure is located ventral to the thalamus, and dorsal to the SN. It receives connections from the cortex, and transmits information to the Gpi (Helie et al., 2013). Dopamine acts on the STN to produce a range of effects, while the STN itself is composed mainly of glutamatergic output. The STN is believed to be a pacemaker region, which emits tonic impulses that coordinate activity in the rest of the BG (Weinberger & Dostrovsky, 2011).

Basal Ganglia Connections

The substantia nigra (SN) and the dopamine system it is part of have a multitude of functions. Dopamine is not the only neurotransmitter of note in the substantia nigra. Dopamine is released often in conjunction with GABA or glutamine (Vogt Weisenhorn et al., 2016; Weinberger & Dostrovsky, 2011). It has also been found that there is significant interaction between adrenergic neurotransmitters and the midbrain dopamine system (Mejias-Aponte, 2016). The dopamine system extends far beyond the SN, it incorporates the various midbrain structures and their projections to the thalamus and to a range of cortical areas (Mejias-Aponte, 2016; Vogt Weisenhorn et al., 2016; Helie et al., 2013). There is also an extensive body of research investigating dopamine's role in other neurological disorders and psychiatric conditions. Dopamine and the basal ganglia have been shown to be involved in PD, Huntington's Disease, schizophrenia, addiction, and quite a few more disorders (Mejias-Aponte, 2016; Vogt Weisenhorn et al., 2016; DeMaagd & Philip, 2015). It is evident that the BG are at a crossroads of many information processing pathways involved in coordination of movement, emotional

expression, reward based learning, and cognitive function (Mejias-Aponte, 2016; Helie et al., 2013).

The ventral mesencephalon has extensive connections to the cortex, striatum, and other surrounding brain areas. The complex web of connections use an array of dopamine, GABA, and glutamate, sometimes with receptors for multiple neurotransmitters at a single synapse (Mejias-Aponte, 2016; Vogt Weisenhorn et al.,

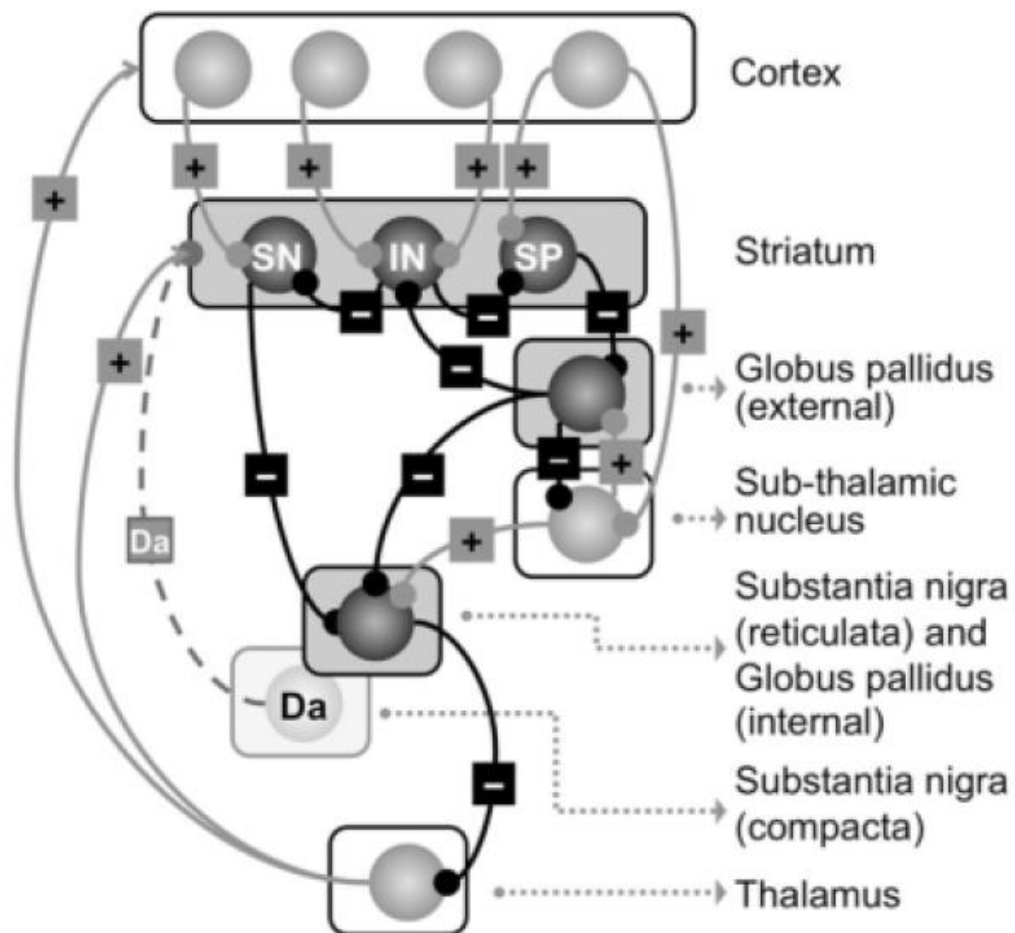


Figure 4 A schematic representation of the circuitry of the basal ganglia. A “+” indicates excitatory connections, “-“ indicates inhibitory connections. Dopaminergic, glutamatergic, and GABAergic connections are shown respectively in light, medium, and dark grey. SP represents the striatopallidal connection, SN represents the striatonigral connection, and IN represents a striatal interneuron. “Da” indicates the dopaminergic connection from the SNc to the striatum. In addition to the information shown in the figure, the dopaminergic projections from the SNc inhibit the striatopallidal pathway, and exert an excitatory effect on the striatonigral neurons. Reproduced from Stocco et al. 2010.

2016). Before delving into the chemistry of the synapses, it is helpful to envision the general layout of the BG neural network in terms of the flow of information. The major input to the BG processing circuit is the striatum and the output is from the Gpi or SNr. Input information originates in the cortex, and output is projected mostly to the thalamus. There are three main pathways which information can take through the BG circuit, called the direct, indirect, and hyperdirect pathways (Helie et al., 2013). In terms of function within the circuit, the SNr and Gpi are often considered one entity, what Stocco (2010) refers to as the *output nuclei*.

The direct pathway transmits cortical information to the Gpi via the striatum. The term ‘direct’ is used because the striatal neurons project directly to the output nuclei, the Gpi and SNr (Stocco et al., 2010). The striatal projections are mostly medium spiny neurons (MSNs). On average, 5,000-10,000 cortical neurons converge on one striatal MSN. The striatonigral MSNs in this pathway express a particular type of dopamine receptor, D1 (Helie et al., 2013).

In the indirect pathway, cortical information is routed through the striatum, then striatal MSNs project to the Gpe, which in turn projects to the output nuclei (Stocco et al., 2010; Weinberger & Dostrovsky, 2011). This pathway is also very convergent, with the same proportion of cortical neurons converging on striatal MSNs as in the direct pathway (Helie et al., 2013). The striatopallidal neurons in this pathway are characterized by expression of dopamine receptor D2 (Helie et al., 2013; Stocco et al., 2010).

The hyperdirect pathway goes from the cortex, to the STN, and ends at the output nuclei. Stocco (2010) considers the STN a second input to the BG circuit because of the

significant quantity of cortical inputs that converge there (Stocco et al., 2010; Weinberger & Dostrovsky, 2011). This indirect pathway is the fastest connection between the cortex and the output nuclei, because it the cortex can interact with the STN over a single synapse (Helie et al., 2013). It is believed that the hyperdirect pathway is responsible for a preliminary and wide ranging inhibition of motor processes, which work in tandem with the dis-inhibitory information from the direct and indirect pathways in order to produce smooth, volitional motion (Nambu et al., 2002).

The three pathways listed here are not the only connections within the BG, although they constitute the majority of the flow of information within the BG circuit. Smaller connections have been found between the various nuclei of the BG, with a decent degree of feedback processes (Stocco et al., 2010). Whereas the three pathways listed above proceed from cortex, to the BG, then to the thalamus, the feedback proceeds in the opposite direction. Some of the more common feedback pathways include Gpe projections to the striatum, STN projections to the Gpe, and connections from the thalamus to the striatum (Stocco et al., 2010; Weinberger & Dostrovsky, 2011). The thalamo-striatal projections synapse on MSNs as well as another prominent striatal neuron class, the tonically active neuron (TAN) (Helie et al., 2013). The TANs are large-bodied striatal interneurons which contribute to plasticity and learning, and are cholinergic. They represent another interface between the dopaminergic system of the BG and other neurotransmitter systems in the brain (Pisani et al., 2001). Another feedback pathway, the nigrostriatal feedback from the SNc to the striatum, is of particular relevance to PD. This route is composed of axons of dopaminergic cells in the SNc and

therefore is one of the most directly impeded pathways in the progression of PD (Stocco et al., 2010).

Dopamine has a regulatory role in the BG and is essential for proper integration of the cognitive and motor processes that result in normal movement. The effect of dopaminergic cell death found in PD pathology has traditionally been explained in terms of the 'rate model' (Weinberger & Dostrovsky, 2011). This model has been a useful guide for PD researchers, as it is able to account for the bradykinesia and the other symptoms which distinguish PD. The premise, as laid out by Weinberger and Dostrovsky (2011), is that dopaminergic inputs from the SN inhibit the striatum. Losing this inhibition leads to striatal hyperactivity, which increases activity of the output nuclei. These output nuclei have a GABAergic inhibitory effect on the thalamus, which leads to thalamic hypoactivity. This essentially makes the thalamic link from the BG to the motor cortex less excitable, resulting in bradykinesia.

While the model has high explanatory power, in reality this is an oversimplification of the system. Research has found that dopamine has a wide range of effects from inhibitory to excitatory, and it can act on both pre- and postsynaptic terminals to affect a different response. To add complexity, dopamine is just one of many neurotransmitter systems which has been identified within the BG (Mejias-Aponte, 2016; Stocco et al., 2010; Vogt Weisenhorn et al., 2016; Weinberger & Dostrovsky, 2011). Furthermore, recent lines of research have explored the possibility that the firing pattern and oscillations of baseline neuronal membrane potential have a more profound effect on BG function than the rate of neuronal firing (Weinberger & Dostrovsky, 2011).

Dopamine Biology

Despite the intricate understanding of the connections within the brain and the effects of depleted dopamine, the underlying pathology which leads to dopaminergic neuronal death is

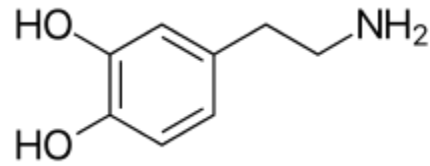
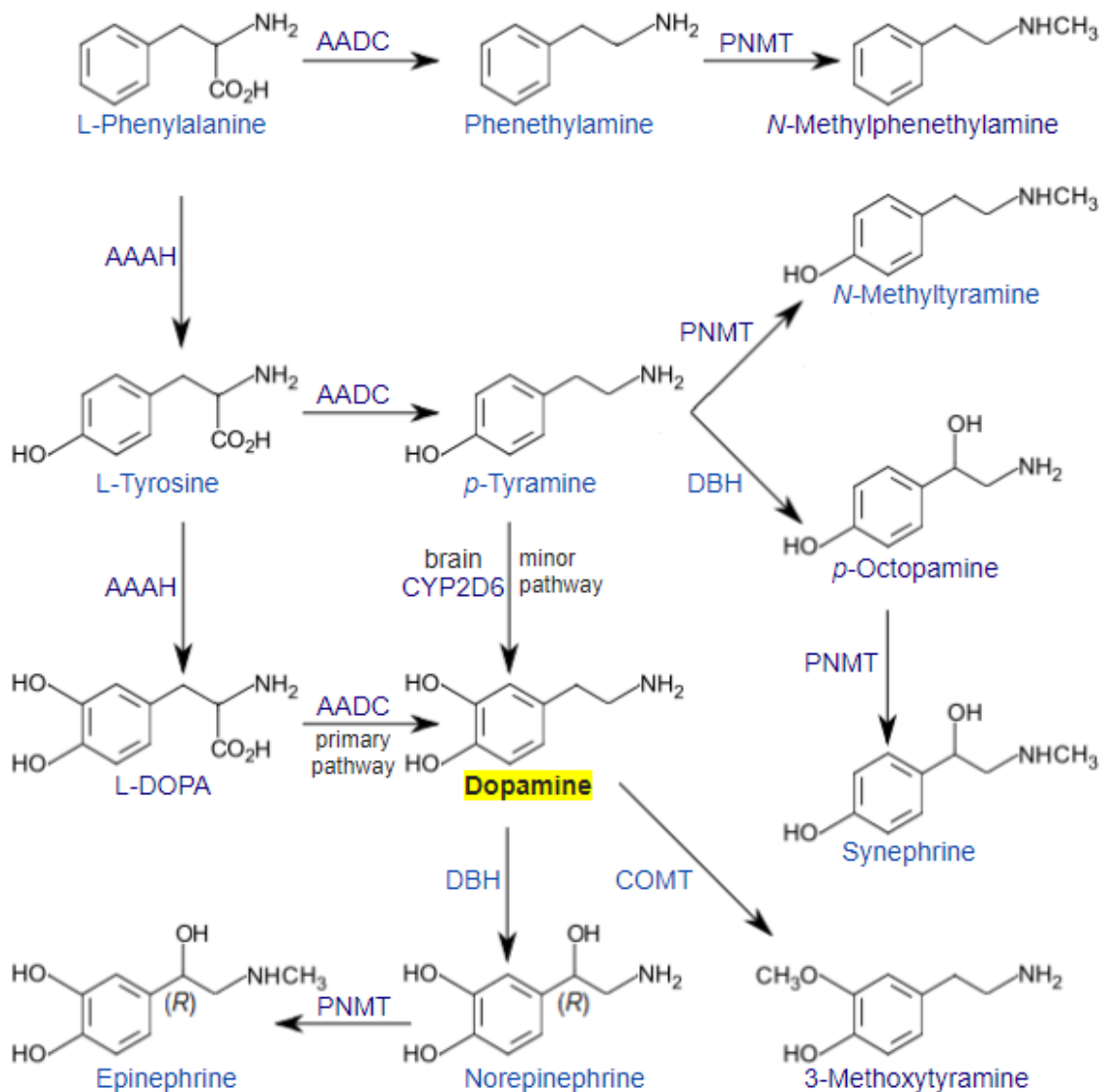


Figure 5 Structure of dopamine. Reprinted from Wikimedia Commons
<https://commons.wikimedia.org/wiki/File:Dopamine.svg>

still poorly understood. Many of the proposed mechanisms involve aggregates of α -synuclein in a pattern similar to Lewy Body Dementia (DLB) and Alzheimer's Disease, although more recently several models have been proposed which involve other pathways to degradation (Plaas et al., 2008; Stuendl et al., 2016). An area which has garnered much attention recently is the effect of oxidative stress in the BG as a result of local cellular biology and dopamine metabolism (Vogt Weisenhorn et al., 2016). It is also likely that there is not a single method of degeneration in PD, but rather the dopaminergic cell toxicity results from a combination of several stressors (DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016).

An understanding of dopamine and the other related neurotransmitters on the molecular level is helpful when envisioning the role of these mediators in the neural microenvironment. Dopamine is a catecholamine, a class of molecules consisting of a 1,2-dihydroxybenzene ring with an amine side chain. Variations in the amine side chain distinguish the three main catecholamines: dopamine, epinephrine, and norepinephrine. These molecules can be interconverted by cellular enzymes (Broadley, 2010).

The dopamine synthesis pathway is a network of enzymes and substrates, which begins with the amino acid L-phenylalanine. This substrate can undergo one of two



reactions. The first, catalyzed by the enzyme aromatic L-amino acid decarboxylase

Figure 6 Dopamine metabolic pathways. Source: Wikimedia Commons
https://commons.wikimedia.org/wiki/File:Catecholamine_and_trace_amine_biosynthesis.png

(AADC), yields phenethylamine, which is then acted upon by phenylethanolamine N-methyltransferase (PNMT) and yields N-methylphenethylamine, a trace amine and isomer of amphetamine. Amphetamine has a wide range of actions in the central nervous system, including addiction and appetite modulation. The second reaction undergone by L-phenylalanine is catalyzed by an aromatic amino acid hydroxylase family enzyme

(AAAH), phenylalanine hydroxylase, yielding L-tyrosine. There are two branches of reactions for L-tyrosine. The first is catalyzed by AADC, converting L-tyrosine to *p*-tyramine. This product is further metabolized into various trace amines, present in insignificant quantities in the human brain (Broadley, 2010). L-tyrosine can interact with a different AAAH enzyme, tyrosine hydroxylase, to produce L-DOPA. Tyrosine hydroxylase is the rate limiting step in the main pathway of dopamine synthesis (Broadley, 2010).

The molecule L-DOPA, also known as levodopa or L-3,4-dihydroxyphenylalanine, is a useful molecule for PD research and treatment. It is able to cross the blood-brain barrier, which dopamine cannot cross. This makes L-DOPA a simple and effective access point to the dopamine synthesis cascade, which allows efficient control over dopamine levels in-vivo. L-DOPA undergoes a decarboxylation reaction catalyzed by AADC to yield dopamine. Dopamine, more formally denoted as 3,4-dihydroxyphenethylamine, is primarily synthesized from L-DOPA by the pathway laid out above, but another pathway exists (Broadley, 2010; Wang et al., 2014).

The *p*-tyramine resultant from the reaction of L-tyrosine catalyzed by AADC is traditionally considered a high-yield source for trace amines, however recent research has found an enzyme localized to the brain which converts *p*-tyramine to dopamine. This enzyme is an isoform of cytochrome P450, labeled CYP2D6. This is a minor synthesis pathway, but has been found to correlate loosely with dopamine depletion in the brain. Wang, Dong, and Yue (2014) report that CYP2D6 expression is 40% lower in the frontal

cortex, cerebellum, and hippocampus of patients with PD compared to unaffected patients. Given cytochrome P450's crucial role in toxicology, it is possible that CYP2D6 has a neuroprotective role, and when its expression is diminished the brain is susceptible to environmental and endogenous neurotoxins (Wang et al., 2014).

There are several metabolic breakdown pathways available for dopamine. Dopamine is a substrate for synthesis of the other catecholamines, epinephrine and norepinephrine. Dopamine reacts with the enzyme dopamine- β -hydroxylase as a catalyst to yield norepinephrine. PNMT then catalyzes a reaction with norepinephrine to produce epinephrine (Broadley, 2010).

Another option is for dopamine to interact with the enzyme catechol O-methyltransferase (COMT), which yields the trace amine 3-methoxytyramine. This amine can react with the enzyme monoamine oxidase (MAO) in conjunction with aldehyde dehydrogenase (ALDH) which produces homovanillic acid (HVA). HVA has no known biological activity; it enters the bloodstream and is filtered out by the kidneys. Both MAO-A and MAO-B isoforms are known to react with dopamine, although there may be a slight preference for dopamine to interact with MAO-B (Broadley, 2010).

The final degradation pathway for dopamine begins with a reaction with MAO that results in DOPAL formation. DOPAL, short for 3,4-dihydroxyphenylacetaldehyde, is believed to be cytotoxic, thus its buildup could be a possible route of neurotoxicity in PD (Broadley, 2010; Miyazaki & Asanuma, 2008). DOPAL undergoes a reaction catalyzed by COMT to produce HVA, which effectively neutralizes the neurotoxic threat of DOPAL (Broadley, 2010).

Dopamine is also potentially neurotoxic. At high levels, dopamine interacts with other dopamine molecules and is oxidized to form DOPA quinones as well as reactive oxygen and nitrogen species. These compounds can harm the cell in which they were produced by interfering irreversibly with protein structure. DOPA quinones have been found to react with proteins, forming 5-cysteinyl-catechols that can hinder protein function. In this way, dopamine-induced oxidative stress can harm cells (Miyazaki & Asanuma, 2008).

The mechanism responsible for neurotoxicity in the dopaminergic neurons of the SNc is still an area of ongoing investigation. Research into the cause of cell death has often dealt with α -synuclein aggregation within cells, and new data shows the involvement of genetic and epigenetic factors such as miRNA silencing and selective expression of transcription factors (Hernandez-Rapp, Rainone, & Hébert, 2017; Vogt Weisenhorn et al., 2016). Researchers are also pursuing consistently reliable biomarkers or other indicators which would indicate definitively whether a patient has PD or not, and also indicate the degree of disease progression (Breen & Lang, 2017).

At present, there still is not a cure for PD. Some speculate that this is due to the high degree of degeneration in the SNc before PD is diagnosed, as if there is a critical threshold of degeneration past which the damage is irreparable and unable to be compensated (DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016). It is essential to detect PD early and initiate therapies before the cell death has progressed past the critical point such that quality of life may be maintained as long as possible (Breen & Lang, 2017; DeMaagd & Philip, 2015).

PUBLISHED STUDIES

The molecular foundation underlying PD is intricately complex. In order to understand the etiology and pathology of PD, it is helpful to understand the microenvironment of the ventral mesencephalon. First this paper will consider heterogeneity of the dopamine system and how it relates to the preferential susceptibility of the SNc. Next the paper will examine the connections within the BG circuitry and explore how their firing patterns influence pathology. Then it will examine the dopamine system's interactions with other neurotransmitter systems in the brain, notably the adrenergic system. The mechanism of dopaminergic neuronal degradation will be considered next, from several perspectives including oxidative stress and α -synuclein aggregation. This section of the paper will conclude by addressing the possibility of early detection of PD via genetic biomarkers and imaging studies.

Heterogeneity of Dopaminergic Subpopulations

The dopamine system which has garnered so much attention for the past few decades is still an active area of research. One of the main questions which researchers are seeking to explain is the asymmetric susceptibility of dopaminergic neurons, i.e. why do the neurons in the SNc degrade while other dopaminergic neurons are nearly untouched. Furthermore, there is disparity within the SNc, where the ventrolateral tier, the nigrosome, is significantly more degraded than the dorsomedial tier (Gibb & Lees, 1991; Vogt Weisenhorn et al., 2016).

Vogt Weisenhorn et al. (2016) provides a thorough account of dopaminergic neuronal differentiation within the ventral mesencephalon. Dopaminergic neurons in the

ventral mesencephalon can be differentiated according to their efferent projection patterns. There are three efferent pathways. Slight overlap exists between the pathways' targets, but the main target of each tract is distinct. The mesostriatal pathway projects to the dorsal striatum, the mesolimbic pathway projects to the ventral striatum, and the mesocortical projects to various cortical targets. The origin of these pathways correlates spatially with the A9 and A10 cell groups. Group A9, localized to the SN, is associated with the mesostriatal pathway, whereas A10 is localized to the VTA and is associated with the mesolimbic and mesocortical pathways. Neurodegeneration is largely limited to the mesostriatal pathway, which further highlights that cellular differences exist within the dopaminergic family (Vogt Weisenhorn et al., 2016).

Differentiation also exists in these dopaminergic neurons' afferent projections. The SN and VTA receive innervation from a wide range of brain structures; notably there is a reciprocal connection between the ventral mesencephalon and the striatum (Weinberger & Dostrovsky, 2011; Yetnikoff et al., 2014). Just as the VTA sends mesolimbic efferents to the ventral striatum, the ventral striatum has afferent projections to the VTA. Similarly, the SNc receives afferent innervation from the striatum. These afferents interact most with the dorsal SNc, but a significant degree of afferents innervate the ventral tier of the SNc as well. In PD pathology, deterioration beyond the SNc is first noted in the lateral striatum, which is predicted by the reciprocal connection along the mesostriatal pathway (Vogt Weisenhorn et al., 2016).

Further differentiation exists among the dopaminergic ventral mesencephalic neurons in terms of their molecular environment. Many of the dopaminergic neurons

exhibit multiplexed neurotransmission, meaning they co-release GABA or glutamate along with dopamine (Mejias-Aponte, 2016). In the VTA, between 5-20% of dopaminergic neurons express the vesicular glutamate transporter-2, whereas this transporter is much less common in the SNc and it is only found laterally (Vogt Weisenhorn et al., 2016).

The neurotransmitter GABA is much more common in the SNc compared with glutamate, which is mostly localized to the VTA (Brichta & Greengard, 2014). The dorsal striatum is replete with GABA induced synaptic currents, thus implying that there is ample GABA activity in the SNc (Vogt Weisenhorn et al., 2016). However, in the vast majority of multiplexed dopamine-GABAergic neurons, neither the vesicular GABA transporter nor GABA synthetic enzymes have been detected (Kim et al., 2015). Vogt Weisenhorn et al. (2016) suggests that GABA is synthesized by a non-canonical pathway, and is released in tandem with dopamine in the same vesicles. The transporter vesicular monoamine transporter-2 is required for GABA release in these cells. In the absence of this transporter, dopaminergic activity is detected but not GABA activity.

As for the synthesis of GABA without the traditional enzymes, Kim et al. (2015) propose a possible synthetic pathway involving aldehyde dehydrogenase 1a1 (ALDH1a1). This is an attractive explanation due to the fact that ALDH1a1 is highly expressed in the dopaminergic neurons in the SNc, and has been shown to be integral to the co-release of dopamine and GABA (Vogt Weisenhorn et al., 2016).

In contrast to GABA, glutamate is not co-packaged with dopamine. It is released at a physically separate space than dopamine and is not contained in the same vesicles as

dopamine. The intricacies of the multiplexed GABA-glutamate-dopamine release patterns allow for the system to be very specific, enhancing the range of dopaminergic signals which regulate complex behavior (Mejias-Aponte, 2016; Vogt Weisenhorn et al., 2016). This view is strengthened by the finding that ALDH1a1, very common in the ventral mesencephalic dopaminergic cells, is intricately tied to rewards pathways that govern alcohol intake and associated addiction behavior (Kim et al., 2015).

Gene expression provides another point for contrasting the nature of midbrain dopaminergic neurons. There is a 97-99% similarity in the gene expression of VTA dopaminergic neurons compared to those in the SNc, which means that 1-3% difference remains and could potentially account for the profound difference in vulnerability of these two neuron populations in PD pathology (Brichta & Greengard, 2014). Differentially expressed genes were found to be involved in a wide range of cellular functions, such as dopamine biology, neuronal activity, neuronal survival, and transcription factors. The genes in each category are not entirely absent in one while present in another cell type, rather the differences in expression are generally related to a strong vs. weak degree of expression (Vogt Weisenhorn et al., 2016).

Poulin et al. (2014) studied 96 genes involved in dopamine biology in the ventral mesencephalon, and used their findings to group dopaminergic neurons into categories. There were two general archetypes, DA¹ and DA², with further subdivisions of DA^{1A}, DA^{1B}, and DA² group A-D (DA^{2A-D}). The categorization of archetypes and subtypes, while based in genetic data, correlated well with the spatial distribution of cells. The DA¹ cluster was localized to the SN, whereas DA² was in the VTA (Poulin et al., 2014).

Within the SN, DA^{1A} was found mostly in the SNc and constitutes the neurons which are most susceptible to degradation in PD pathology (Gibb & Lees, 1991; Vogt Weisenhorn et al., 2016). A unique finding of subset DA^{1A} is that it expresses the highest levels of Kcnj6 (aka Girk2), a G-protein regulated potassium channel. It is possible that the selective degeneration in this region is linked to expression of this channel (Poulin et al., 2014).

The Kcnj6 channel is related to neural activity regulation. In a study done with mice, researchers found that mice with a homozygous knockout of Kcnj6 began losing SNc neurons on day 7 post-natal, and at 90 days had lost almost 70% of the SNc dopaminergic neurons (Brichta & Greengard, 2014). It seems contradictory that in mice without this gene there was significant degeneration, while high expression of this channel is also linked to degeneration as found in Poulin et al. (2014). This area is of interest to future research.

Another interesting finding from this line of research is that DA^{1A} subtype is characterized by high expression of ALDH1a1. ALDH1a1 is believed to have a neuroprotective effect, as it oxidizes the neurodegenerative dopamine metabolite DOPAL into HVA (Broadley, 2010). High DOPAL concentration has been shown to promote α -synuclein aggregate formation, which is effectively prevented by ALDH1a1. However, these cells in the SNc are still especially vulnerable in PD, so there must be other patterns of degeneration involved which overpower the ALDH1a1 neuroprotective effect (Vogt Weisenhorn et al., 2016).

Whereas DA^{1A} was found to have a unique feature conferring toxicity, DA² was found to uniquely express the transcription factor Otx2. The study authors found that Otx2-expressing cells mitigated the toxic effect of MPTP, a known neurotoxic compound. In contrast, DA^{1A} cells were susceptible to MPTP induced toxicity (Anderegg et al., 2015). The DA² group was also found to express Fzd1, a gene which encodes a receptor in the Wnt1 cascade. Fzd1 is another neuroprotective agent which protects cells from cytotoxins as well as protects against degeneration if certain transcription factors are lost, e.g. En-1. Fzd1 is not expressed in appreciable levels in the DA^{1A} subtype (Poulin et al., 2014).

The post-translational modification of proteins differs between populations of dopaminergic neurons in the ventral mesencephalon. There is not much written in the literature about this phenomenon, so it merits future study. However there are some preliminary studies which indicate that the dopamine transporter Slc6a3 is glycosylated quite often in the SNc, while the glycoprotein is less common in the VTA (Vogt Weisenhorn et al., 2016). The glycosylated form is highly active and is present in about 20% of SNc dopaminergic neurons (Brichta & Greengard, 2014).

The genetic findings of Poulin (2014) are useful in differentiating dopaminergic subtypes, but they fall short of providing conclusive evidence for a genetic marker of PD. The genes which are traditionally associated with PD pathology (α -synuclein, Parkin, Pink1, Lrrk2, etc.) were shown to be ubiquitously expressed throughout the ventral mesencephalon, and could not distinguish a unique subpopulation (Poulin et al., 2014). Therefore, it is unlikely that that a single gene mutation could be linked to the

degradation pattern characteristic of PD. This has led some researchers to propose a multiple-hit hypothesis among the genes, where no single mutation would contribute to degeneration but several in conjunction could have deleterious effects (Vogt Weisenhorn et al., 2016).

Basal Ganglia Circuit

The overt motor symptoms of PD are rooted in the heterogeneity of the dopamine system. The classic conception of PD pathology was that the loss of dopaminergic inhibition in the striatum led to hyperactivity, which would increase the rate that the striatum sent information to the SNr and Gpi. The output nuclei tonically inhibit the thalamus via GABA action, so an increase in stimulation from the output nuclei leads to a decrease in thalamic excitability. The end result is that the thalamus relays less information to the motor cortex, which gives rise to bradykinesia (DeMaagd & Philip, 2015; Weinberger & Dostrovsky, 2011; Yetnikoff et al., 2014).

Weinberger and Dostrovsky (2011) propose a slightly different version of this model which generates the motor symptoms of PD. This model, based on data from electrophysiological recordings of the cortex and BG circuit in both animal models and human patients, focuses on the alterations in firing patterns and baseline oscillations in membrane potential of neurons which occur secondary to chronic dopamine depletion. Electrodes were implanted in patients' brains which offered therapeutic regulation to patients, and allowed researchers to manipulate the local field potentials at the implantation location, i.e. the STN and Gpi. Animal models were constructed where monkeys were treated with MPTP and rats were treated with 6-hydroxydopamine to

artificially degrade the SNc and record the results with electrodes. In a dopamine depleted state, there is increased functional connectivity between the cortex, STN, and globus pallidus. It seems that increased synchrony in neuronal firing, and increased oscillatory firing within the BG contribute significantly to PD pathology (Weinberger & Dostrovsky, 2011; Yetnikoff et al., 2014).

Chronically depleted dopamine levels in the striatum lead to a surfeit of synchronized oscillatory firing within the BG-cortical loops. This oscillation can be observed and induced in vivo in PD patients who withdraw from anti-parkinsonian medications (Stocco et al., 2010; Weinberger & Dostrovsky, 2011; Yetnikoff et al., 2014). The oscillations occurred most prominently in the STN and globus pallidus, and align with one of two frequencies (Weinberger & Dostrovsky, 2011). The tremor frequency is around 4-6Hz (DeMaagd & Philip, 2015), and takes precedence in PD patients whose main symptom is tremor, whereas the beta frequency is around 11-35Hz. The beta frequency was also observed in rats in the 6-hydroxydopamine model. These effects can be rapidly reversed by administering dopamine medications. In the dopamine depleted state, MSNs express a higher degree of gap junctions which electrically couple them to neighboring cells, facilitating synchronization of firing. The exact mechanism that links synchronization to pathology is uncertain, but the observed alteration in firing pattern is unique to neurons undergoing PD degeneration (Weinberger & Dostrovsky, 2011).

MSNs in the striatum are more excitable in the absence of dopamine. Under normal conditions, their membrane potential is very polarized. The potential is

determined by inward movement of rectifying potassium currents which maintain a hyperpolarized state for the membrane. Action potentials can only occur when the summation of concurrent impulses overcome the rectifying current and cross the neuron's threshold. Dopamine inhibits MSN excitability by promoting the hyperpolarization of the MSN membrane (Weinberger & Dostrovsky, 2011).

Altogether, there are 5 main isoforms of the dopamine receptor, D1-5, and each has a marginally different effect on cellular function. The distribution of these receptors in the BG is not uniform (Brichta & Greengard, 2014). Dopamine induces its inhibitory effect through interaction with the D1 receptor. D1 is found on the post-synaptic terminus of striatal MSNs and is a G-protein coupled receptor (GPCR). Studies have found that antagonists of this receptor effectively silence the MSN. Dopamine has an additional inhibitory effect on the presynaptic terminus of neurons projecting to the striatum, where dopamine inhibits glutamate release by interacting with the D2 receptor. Loss of dopamine reduces this striatal inhibition by both the D1 and D2 receptor, allowing a much greater proportion of cortical input to enter the BG circuit (Weinberger & Dostrovsky, 2011).

In the Gpe, dopamine has an excitatory effect. This is accomplished via interaction with presynaptic D2 receptors on striatopallidal terminals, which works to inhibit the release of GABA. Dopamine has a postsynaptic action here as well, mediated by the D4 receptor, which suppress GABA-induced currents. In the Gpi and SNr, dopamine has an opposite effect. It activates presynaptic D1-like receptors, which induces greater GABA release and reduces Gpi neuronal activity. While the action of

dopamine in the Gpe and Gpi are seemingly incongruous, it appears that the overall action is one that inhibits transfer of cortical inputs. In the pathological state, the dopaminergic inhibition decreases and there is a higher degree of connectivity (Weinberger & Dostrovsky, 2011).

The STN is a pacemaker for impulses in the BG. It is dense with glutamatergic neurons that can generate action potentials without synaptic input. It is believed that this is caused by voltage-gated sodium channels (Yetnikoff et al., 2014). Activation of D1 and D2 receptors in the STN encourages tonic firing patterns in this region. In the normal state this neuron population has a high degree of tonic firing, with little burst activity, a finding which is reversed in PD pathology. The sodium channels which allow for tonic firing are inactivated by autonomous firing in the STN, and require feedback from the Gpe to reactivate these channels. This feedback loop is thrown off balance in the absence of dopamine, and the synchronized pattern of pathological firing results (Weinberger & Dostrovsky, 2011).

The excitatory STN input to the Gpe under normal conditions constitutes the main control factor for Gpe firing, with inhibitory striatal input taking secondary prominence. In the dopamine depleted state this input pattern switches, and striatal inhibition is the main input to the Gpe. This contributes to the irregular firing patterns characteristic of PD (Weinberger & Dostrovsky, 2011).

The data from PD patients confirms the findings of Weinberger and Dostrovsky (2011). Measured by deep brain electrodes and electroencephalogram (EEG), PD patients show a high degree of synchronized firing in the STN and Gpi, with notable burst firing

in each nucleus at the beta frequency (11-35Hz). This effect is greatly reduced by anti-parkinsonian medications. The inhibition afforded by dopamine is essential for proper functioning of the system; in its absence the synchronized oscillations of the BG firing patterns impede proper processing of motor signals (Weinberger & Dostrovsky, 2011).

Interface of Adrenergic and Dopamine Systems

In addition to the GABAergic and glutamatergic connections outlined above, the dopamine system is very closely tied to the midbrain adrenergic system. Mejia-Aponte (2017) succinctly outlines the interface between the two, and the implications of adrenergic modulation in dopaminergic dysregulation. Epinephrine and norepinephrine are abundant neurotransmitters in the brain, and act through several adrenoceptors. In general, α_1 , and β_{1-3} adrenoceptors are considered excitatory, whereas α_2 and occasionally β_2 are inhibitory. All the receptors are GPCRs. Developing an understanding of the function of this system could give insight to possible routes of future treatment, or compensatory mechanisms available in the brain as dopamine degrades.

The adrenergic innervation of the ventral mesencephalic dopamine complex arises in the brainstem adrenergic nuclei and in the locus coeruleus. Norepinephrine afferents originate in cell groups A1 and A2, which correspond to the caudal ventrolateral medulla and the solitary tract nucleus, respectively. Epinephrine afferents come from the cell group C1, located in the rostral ventrolateral medulla (Dahlstroem & Fuxe, 1964). Most of the adrenergic axons innervating the dopaminergic system interact via classical synapses, with a lesser proportion interacting via glia processes, and a smaller proportion with gap junction interfaces. The adrenergic axons also interact with non-dopaminergic

neurons in the ventral mesencephalon although these associations are yet uncertain in function. In the SN, the dopaminergic and non-dopaminergic neurons are clearly delineated, but in the VTA it seems they are not segregated (Mejias-Aponte, 2016).

The ventral mesencephalon dopaminergic neurons express the excitatory α_1 adrenoceptors most prominently. Analysis of mRNA in these cells found little evidence for α_2 and β_2 expression. However, experimental techniques with autoradiography and immunohistochemistry found evidence for both α and β receptor expression in this region. The full range of adrenoceptors has been found in the ventral mesencephalic dopaminergic neurons, albeit not all by a single technique. It is still uncertain whether the adrenoceptors are localized to the pre- or postsynaptic terminal, or if their distribution is general. In dopaminergic neurons, α_1 receptors have been found in both glutamatergic as well as GABAergic terminals, which is indicative of pre-synaptic adrenergic modulation of these neurotransmitters' signaling (Velásquez-Martínez et al., 2015). Similarly, α_2 adrenoceptors have been found in the VTA and SN. The effect of this receptor is generally post-synaptic, but this has not been conclusively confirmed by research. The α_2 receptors found on non-dopaminergic neurons in the ventral mesencephalon indicate a possible route by which adrenergic inputs can indirectly regulate dopaminergic neurons via a non-dopaminergic modulator (Mejias-Aponte, 2016).

The adrenoceptors are not unique to neurons. Adrenoceptor α_1 and the norepinephrine transporter (NET) have both been observed in glia. Studies have also found α_2 and the β receptors expressed in microglia and astrocytes in the midbrain. This finding is of interest, because glia are known to play a role in maintain the apposition of

the synaptic cleft in noradrenergic terminals. Further research is needed to elucidate the role of glia in midbrain norepinephrine biology (Mejias-Aponte, 2016).

Under normal conditions, midbrain dopaminergic neuron impulses occur at regular intervals with occasional burst patterns. The bursts of action potentials effectuate a large-scale release of dopamine at the axon terminals. These bursts are often a response to behavioral circumstances, such as a response to a conditioned stimulus (Friston et al., 2014; Yetnikoff et al., 2014). Several adrenergic drugs have been shown to alter the firing patterns of the dopaminergic neurons. For example, the α_2 agonist clonidine did not affect the firing rate of DA neurons but it increased firing regularity. Idazoxan and piperoxine, on the other hand, both interact with α_2 to antagonize the effects of clonidine and increase occurrence of burst firing. As for decreasing the firing rate, prazosin and isoprotenerol were shown to accomplish this via the α_1 and β adrenoceptors, respectively (Mejias-Aponte, 2016).

Three neuromodulation mechanisms were described for the interaction of norepinephrine and glutamate release. Norepinephrine was observed to potentiate glutamate release, in that it increased the overall trend of glutamate release independently of the spontaneous activity of the neuron. In other cases, norepinephrine enhanced glutamate release, causing larger quantities of the neurotransmitter to be secreted. Lastly, norepinephrine was noted to have a glutamate suppression character as well, decreasing the total release quantity. The exact receptors responsible for each effect have not been determined, but it appears that the excitation modulation is sensitive to α_1 receptor agonists, and suppression was insensitive to α_1 , α_2 , and β adrenoceptor antagonists.

Furthermore, electrical stimulation of the A2 and C2 cell groups induced a response in the ventral mesencephalic dopaminergic neurons, and the response involved both inhibitory and excitatory elements (Mejias-Aponte, 2016).

In ex-vivo conditions, norepinephrine was found to have an excitatory effect on dopaminergic neurons. Acting through the α_1 receptor, norepinephrine elicited depolarization in 36% of dopaminergic neurons, which led to an increase in firing rate. This effect can be reproduced with the adrenergic drug phenylephrine, an α_1 agonist, and inhibited by prazosin, an α_1 antagonist (Mejias-Aponte, 2016).

The α_1 receptor appears to be involved in two ion currents' regulation. Activation of the receptor decreases calcium-activated potassium channel currents in the dopaminergic neuron, while it increases hyperpolarization-activated cation currents. Altogether, this receptor evokes an increase in burst firing (Mejias-Aponte, 2016).

There is evidence of presynaptic norepinephrine action via the α_1 receptor. Activation of the receptor triggers a decrease in the frequency of spontaneous GABA-induced postsynaptic currents. This mechanism uses a phosphokinase-C signaling pathway, and is dependent on the activation of calcium and voltage gated potassium channels. Presynaptic activation of the α_1 receptor was also noted to evoke glutamate release (Velásquez-Martínez et al., 2015). Both of these findings indicate that presynaptic adrenergic activation of the α_1 receptor excites the dopaminergic neuron, and when glutamate and norepinephrine both interact with a single dopaminergic neuron, that neuron will fire longer bursts of action potentials (Mejias-Aponte, 2016).

Norepinephrine exposure quickly builds desensitization of the α_1 receptor, so the excitatory effects are often short bursts and are less apparent when norepinephrine levels are tonically high, as in times of stress. This desensitization effect is apparently blocked by some stimulant drugs, notably cocaine and amphetamine (Broadley, 2010; Mejias-Aponte, 2016). This represents yet another connection between the dopamine system and other behavioral pathways beyond PD, such as reward seeking behaviors and addiction (Friston et al., 2014; Mejias-Aponte, 2016; Velásquez-Martínez et al., 2015).

The adrenergic system plays a role in regulation of dopamine levels. In studies where the locus coeruleus is lesioned, the tonic dopamine levels elsewhere in the brain were decreased. Agents known to inhibit the breakdown of norepinephrine and agonists of norepinephrine release were both found to correlate with higher dopamine levels in the midbrain. The structural similarity between the catecholamines allows for a degree of cross-talk between receptors and ligands. Norepinephrine is known to interact with the D2 and D4 receptors on dopaminergic neurons, albeit with lesser affinity than dopamine, which produces an inhibitory effect in neuronal activity (Mejias-Aponte, 2016). This effect is not surprising, as dopamine and norepinephrine differ by only one hydroxyl group in on the alpha carbon associated with the amine (Broadley, 2010). The adrenergic system therefore is an interesting route for future research to explore therapies aimed at increasing tonic dopamine levels in the midbrain.

Mechanisms of Degradation

It is a widely-accepted fact that the dopaminergic neurons in the ventral mesencephalon degrade in PD pathology, but the mechanism by which it occurs is not as

well established (DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016). The numerous proposed mechanisms of neurotoxicity span many disciplines, however, this paper will focus on several molecular approaches. The protein α -synuclein is known to play a role in the pathology of many neurodegenerative diseases, and PD is no exception (Braak et al., 2003). This protein aggregate is undoubtedly neurotoxic, but there are other factors involved in PD pathology which must also be considered. These include the high oxidative stress present in certain midbrain cells and the neurotoxicity implicit in dopamine's biochemical pathways, and the deleterious effect of neuroinflammation (Graumann et al., 2002; Miyazaki & Asanuma, 2008).

the Braak Hypothesis and α -synuclein

Braak et al. (2003) proposed that neurodegeneration in PD is related to the spread of a pathogen, in a manner similar to a prion. They believe that sporadic PD, i.e. not the familial genetic variant, is caused by an unknown pathogen, which enters the body through the nasal cavity and is ingested, then absorbed in the gut and in the nasopharynx. The pathogen travels along the olfactory tract and enteric vagal nerve towards the central nervous system, as evident by the spread of α -synuclein aggregates which track the pathogen's migration. The pattern of spreading both systemically and within the central nervous system is predictable and, as Braak et al. (2003) believe, contribute to a staging pattern which could ideally be used to quantitatively determine progression of PD. The spinal cord does not express α -synuclein aggregates until after the CNS is already involved, so the spinal cord therefore does not represent a potential route for this

pathogen to travel rostrally. This conception of PD is referred to as Braak's hypothesis (Rietdijk et al., 2017).

The involvement of the gut at an early stage of the disease is an astute observation on behalf of Braak et al. (2003). PD patients often report GI and olfactory symptoms before a diagnosis of PD is made, such as constipation, dysphagia, nausea, and anosmia. This data corresponds to findings of Lewy bodies in the GI tract and olfactory tract of PD patients (Rietdijk et al., 2017). Lewy bodies are neurofibrillary aggregates composed mostly of α -synuclein which are implicated in several neurodegenerative diseases, such as PD, Lewy Body Dementia, and parkinsonian multiple system atrophy (DeMaagd & Philip, 2015). There is a positive correlation between the level of Lewy body aggregates in the enteric nervous system and the severity of gastrointestinal symptoms (Braak et al., 2003). PD is not diagnosed until motor symptoms are present, since the classic triad of motor symptoms is a key distinguishing feature of PD; this pre-diagnosis period is referred to as Incidental Lewy Body disease (Rietdijk et al., 2017).

Within the gastrointestinal tract there are several differences between PD patients and controls. Disease-free control subjects had higher levels of certain microbiome flora compared to PD patients, notably in the abundance of prevotellaceae bacteria, whereas PD patients had significantly higher enterobacteriaceae. High level of enterobacteriaceae was associated with more severe postural instability and gait symptoms and less pronounced tremor, when compared within the PD patient subset. PD patients also exhibit greater intestinal permeability, a hallmark sign of intestinal inflammation. This is likely due to increased bacterial intrusions into the mucosal epithelia, oxidative stress,

and α -synuclein deposition. It is possible that the changes in gut flora predispose the patient to PD risk (Rietdijk et al., 2017).

Patients' diets could play a role in susceptibility. Fiber-rich diets promote growth of flora that produce short-chain fatty acids which mitigate inflammation, whereas diets high in fat and processed carbohydrates lead to a balance of gut flora that is more inflammation-prone (Rietdijk et al., 2017). The link between diet and PD is not confined to bacteria. It appears that high-iron diets are also correlated with risk of PD due to the deleterious effects of storing metals in cells long-term (Hare et al., 2017; Tribl et al., 2009).

The spread of the pathogen suggested in the Braak hypothesis follows the vagus nerve. It originates in the enteric nervous system, then ascends the vagus until it reaches the dorsal motor nucleus of the vagus in the medulla, then enters the central nervous system. There it spreads to the brainstem, then the midbrain including the SN, and upward to the neocortex (Rietdijk et al., 2017). This hypothesis accounts for the general trajectory of α -synuclein pathology, but Rietdijk et al. (2017) point out that the α -synuclein pathology spread is not all-encompassing. The structures listed are renowned for their susceptibility, but neighboring structures are virtually untouched. They use the example of the dorsal motor nucleus of the vagus, which exhibits Lewy body pathology, neighbors the solitary tract nucleus, which is spared. In animal models it has been shown that vagotomy is an effective measure to stop spread of α -synuclein to higher parts of the nervous system, but the practicality of this in humans is uncertain and should be considered on a case-by-case basis (Rietdijk et al., 2017).

The neurons which are most susceptible to α -synuclein pathology have some features in common. They have a high baseline metabolic rate, which contributes to increased oxidative stress and α -synuclein misfolding. Additional factors are that they have high endogenous levels of α -synuclein and use monoamine neurotransmitters, their axons are long, branched, and have little to no myelination, and they are almost always constitutively active. It is possible that α -synuclein pathology travels from the peripheral to central nervous system along a path of least resistance, traversing up interconnected populations of highly susceptible neurons while the neighboring protected neurons are left untouched (Rietdijk et al., 2017).

The protein α -synuclein's neurotoxicity is similar to that of a prion. The natural state is a protein of unknown function which is ubiquitously expressed in the brain and some peripheral tissues. This form is not believed to be inherently neurotoxic. Pathogenic seed particles are then introduced, either sporadically via mutation or from ingestion as in the Braak hypothesis. Stuenkel et al. (2016) found that the pathogenic particles are prone to oligomerization, and interact with natural state α -synuclein to coax it into the pathogenic form. Furthermore, the α -synuclein isolated from exosomes in the cerebrospinal fluid (CSF) was preferentially in an oligomerized state, and could convert nascent α -synuclein from other samples into the oligomer form (Rietdijk et al., 2017; Stuenkel et al., 2016). Therefore it appears that the oligomers of α -synuclein are pathogenic, and in high numbers these aggregates form Lewy bodies. Oligomeric inclusions of α -synuclein in neurons interfere with cellular processes, and reduce network

connectivity. In large enough quantities, this interference of the Lewy body with cellular function causes cellular stress unto death (Rietdijk et al., 2017).

Unfortunately, the Braak hypothesis falls short of its goal to provide accurate staging for PD progression. In the subset of PD patients studied, between 51-83% follow the Braak staging patterns. About 7-11% do not express Lewy bodies in the dorsal motor nucleus of the vagus, while higher areas in the central nervous system are affected, a result incongruous with the staging hypothesis. 27-33% of PD patients had olfactory α -synuclein aggregates, but none in the enteric nervous system, again contradicting the hypothesis which states that patients should have α -synuclein in both the olfactory and enteric pathways. These findings could be explained if α -synuclein pathology developed at multiple, sporadic locations. While the hypothesis is applicable to a majority of PD patients, it is not an all-inclusive diagnostic, but rather a guideline to be followed cautiously. Patients who were diagnosed with PD at a relatively young age, have a long disease course, and whose primary symptoms are motor, not dementia, align the closest with Braak's hypothesis (Rietdijk et al., 2017).

There is a further issue of timing of disease progression. Kordower (2013) found that most neurons in the SNc of PD patients have degraded within 4 years after diagnosis, and subsequent loss after this is incidental. Loss after 4 years could represent the loss of a compensatory mechanism or loss of non-dopaminergic neurons (Vogt Weisenhorn et al., 2016), or it could be normal aging (Isaias et al., 2016; Kordower et al., 2013). Even in the earliest time points post-diagnosis, brains of PD patients examined post-mortem showed 50-90% degradation in neurons expressing tyrosine hydroxylase, the catalyst of the rate

limiting step of dopamine synthesis, with subsequently marginal loss up to 4 years post diagnosis (Kordower et al., 2013). Braak's hypothesis does not mention timing of α -synuclein spread, so it is hard to tell if α -synuclein invades the SNc while it is healthy and causes degeneration, or if the Lewy body pathology only infringes upon the SNc once the neurons have already degenerated due to other means (Rietdijk et al., 2017; Stuenkel et al., 2016).

Oxidative Stress

Another mechanism of cellular toxicity which has garnered much attention is that of oxidative stress. SNc neurons are densely packed with mitochondria, and have 3-fold greater respiratory energy demands than VTA cells at baseline (Vogt Weisenhorn et al., 2016). This high metabolic rate generates a similarly high degree of reactive oxygen species (ROS), reactive nitrogen species (RNS), and is conducive to the auto-oxidation of dopamine (Miyazaki & Asanuma, 2008). Altogether, these byproducts of oxidative respiration in cellular metabolism cause inflammation and pose serious threats to the integrity of the cells which contain them (Crotty et al., 2017).

The brain is predisposed to oxidative stress. The brain accounts for 2% of the body's weight, yet its metabolic rate consumes about 20% of the body's total oxygen supply and a significant portion of that oxygen is converted into ROS (Crotty et al., 2017). A significant portion of the oxidative stress in the ventral mesencephalon is due to dopamine. Under normal conditions, dopamine levels are regulated and the neurotransmitter is packaged in vesicles, but extravesicular dopamine in high concentration is able to react in many different ways (Miyazaki & Asanuma, 2008).

Therapy with L-DOPA is the most common treatment for PD, but long term treatment can have deleterious effects. L-DOPA therapy introduces high levels of exogenous dopamine in cells which reacts in ways that generate ROS and RNS. In advanced PD, the SNc is degraded to a point where so few dopaminergic neurons remain that L-DOPA is unable to elicit an appreciable effect. In both of these cases there is a surplus of dopamine in the microenvironment which cells process in a variety of ways that pose a risk in terms of oxidative stress (Miyazaki & Asanuma, 2008).

Some ROS result from the normal degradation pathway of dopamine. The enzyme MAO converts dopamine into dihydroxyphenylacetic acid (DOPAC) and produces H_2O_2 as a byproduct. The H_2O_2 in dopaminergic neurons has been shown to interact with metals, producing the hydroxide (OH^-) ion. The hydroxide ion is very reactive and constitutes a major risk to cellular processes. Iron has the apparent highest affinity for interaction with hydrogen peroxide (Miyazaki & Asanuma, 2008). This finding is interesting, because iron-rich diets early in life are a risk factor for developing PD pathology later in life (Hare et al., 2017).

The quinones and free radicals which result from non-enzymatic auto-oxidation of dopamine are implicated in neurotoxicity. In an oxidative environment, dopamine spontaneously oxidizes, generating quinones and superoxide ions, O_2^{1-} . The enzyme superoxide dismutase converts O_2^{1-} into hydrogen peroxide, which is less cytotoxic but still harmful to cells, as described above. Superoxide ions also interact with nitric oxide radicals, NO^\cdot , yielding the RNS species peroxynitrite ($ONOO^-$). Free radicals, ROS, and RNS species are highly cytotoxic. They disrupt protein structure, cause stress to cells, and

result in inflammation (Crotty et al., 2017; Miyazaki & Asanuma, 2008). Whereas ROS and RNS exercise a harmful effect on the neuron in which they are made as well as neighboring cells in the local environment, the deleterious effects of quinones are often limited to the neuron which produced them (Fedorow et al., 2005; Graumann et al., 2002; Miyazaki & Asanuma, 2008).

Structurally, quinones formed from catecholamines have ketone groups where the hydroxyl groups were in the catechol form (Pubchem, n.d.). The quinones produced in the auto-oxidation of dopamine have a wide range of cellular effects. Miyazaki and Asanuma (2008) find that dopamine quinones are also generated from dopamine oxidation by enzymes with cyclooxygenase activity, such as prostaglandin H synthase, lipoxygenase, tyrosinase, and xanthine oxidase. L-DOPA is also oxidized to a quinone form without being converted to dopamine first, and its consequences are similar to the observed effects of its dopamine counterpart (Miyazaki & Asanuma, 2008).

Dopamine quinone reacts covalently with cysteine. The cysteine residue is often found in the active site of proteins, so covalently binding dopamine effectively inhibits protein function (LaVoie & Hastings, 1999; Sulzer & Zecca, 2000). Glutathione, an enzyme known for alleviating oxidative stress, has a prominent cysteine residue which is targeted by dopamine quinones. This exacerbates the toxicity of quinones and stresses associated with a highly-oxidizing environment by degrading this essential neuroprotective antioxidant (LaVoie & Hastings, 1999).

The oxidative stress caused by dopamine metabolite have several key implications in the midbrain dopaminergic system. Dopamine quinones interact with cysteine residues

in tyrosine hydroxylase and render it inactive (Miyazaki & Asanuma, 2008). This enzyme catalyzes the rate-limiting step of dopamine synthesis (Broadley, 2010), thus inactivation of the enzyme may lead to lower dopamine levels. Dopamine quinone has also been found to bind with cysteine residues in the dopamine transporter DAT, preventing dopamine uptake at the synapses (Miyazaki & Asanuma, 2008). The dopamine quinones have an important action regarding the protein product of one of the few PD-associated mutant genes, the parkin protein, causing it to be less soluble and inactivating its ubiquitin ligase function (LaVoie & Hastings, 1999; Sulzer & Zecca, 2000). This has been linked to apoptotic degenerations in dopaminergic neurons, and mutant parkin expression is positively correlated with presence of aminochrome (Crotty et al., 2017). Furthermore, dopamine quinones have been shown to conjugate with α -synuclein. This promotes aggregation of the insoluble, pathogenic form of the protein, and contributes greatly to the formation of Lewy bodies. By decreasing solubility of α -synuclein, the catecholamine-derived quinones promote the destruction of synaptic vesicular membranes (Miyazaki & Asanuma, 2008).

Dopamine and L-DOPA quinones are a key component to the formation of neuromelanin. The quinones are further oxidized to cyclized aminochromes, either enzymatically or because of the oxidative environment. Dopaminochrome and L-DOPA-chrome both polymerize, and are a major component of the pigmented neuromelanin aggregates in neurons (Miyazaki & Asanuma, 2008). Interestingly, neuromelanin is found in only two of the three cell catecholaminergic types in the brain: dopaminergic and noradrenergic cells express neuromelanin, while adrenergic ones do not.

Neuromelanin is not expressed in non-catecholaminergic cell types (Fedorow et al., 2005).

Fedorow et al. (2005) also observe that whereas the dopaminergic and noradrenergic neurons are capable of expressing neuromelanin, not all cells in these lineages do in fact express it, so neuromelanin expression cannot be used as an exact quantification of neurodegeneration. Even so, the brains of PD patients when examined post-mortem are uniformly lacking in pigmentation (Kordower et al., 2013; Vogt Weisenhorn et al., 2016). Research has also shown that the highest concentration of neuromelanin in the brain is in the dopaminergic neurons of the ventral mesencephalon, in particular the SN (Fedorow et al., 2005).

It is believed that neuromelanin has a neuroprotective function in the cell. An inverse relationship was established between neuromelanin expression and vulnerability in PD pathology (Hirsch et al., 1988). This may be due to the tendency of neuromelanin to aggregate the harmful metabolites of dopamine and neutralize their oxidant behavior (Fedorow et al., 2005; Miyazaki & Asanuma, 2008; Sulzer & Zecca, 2000). Accordingly, an investigation of cells that MRI images showed to be high in neuromelanin were found to have similarly high amounts of binding to the dopamine D2 receptor, confirmed by PET scan (Ito et al., 2017).

Neuromelanin plays a role in neutralizing potentially toxic metal cations. Iron, zinc, lead, copper, manganese, cobalt, mercury, and other metals can damage cells in high concentrations, especially in the oxidative environment within the cell. In particular, iron has become an area of interest for PD research. There is evidence linking iron

accumulation in the SN to PD disease progression. Neuromelanin-expressing cells seem to resist metal cation damage more robustly compared to cells without pigment (Fedorow et al., 2005; Tribl et al., 2009).

Neuromelanin comprises one aspect of the cellular neuroprotection from oxidative stress, and it is attractive to PD researchers because of its direct involvement in the metabolism of dopamine. Other neuroprotective systems exist within the cell, albeit not as intricately tied to dopamine metabolism. One such system involves purine metabolism (Crotty et al., 2017). It would be worthwhile to explore the connections between the multitudinous systems of protection available to the dopaminergic neuron in future research.

Towards Early Detection

As there is not yet any cure for PD, it is imperative to detect the disease early. Dopaminergic neurons are lost exponentially more rapidly in the early part of disease progression than in the advanced stages (Kordower et al., 2013), so treatments initiated earlier will likely be able to protect many more neurons and preserve brain function for a longer period (Breen & Lang, 2017; DeMaagd & Philip, 2015). Towards this goal, there is a wealth of research examining the possibilities of PD-linked biomarkers and signals in imaging of PD brains.

Breen and Lang (2017) highlight that the cognitive and motor symptoms of PD appear far earlier than the diagnosis is made. There is a trend among clinicians to observe symptoms and monitor their progression post-onset until they conclusively confirm or rule out PD, referred to as the ‘wait and watch’ approach. Given the early onset of

symptoms, it would be worthwhile to develop a measure to detect PD earlier in the prodromal stage (Breen & Lang, 2017).

Darweesh and colleagues (2016) found some surprising results about the time frame of prodromal PD. On cognitive tests, PD patients began to show significantly greater decline in performance compared with control subjects about seven years prior to PD diagnosis. This correlated with data showing that patients reported having difficulty with complex tasks involving motor and non-motor function, especially traveling, about seven years pre-diagnosis. Patients reported that their motor symptoms first began to interfere with daily life about five years before a diagnosis was made, particularly with regard to eating, and the gastrointestinal dysregulation and tremor noted in PD could be detected up to ten years prior to diagnosis. Motor impairments onset in the upper limbs, generally as finger tapping or arm swinging, and then progress into tremor about five years prior to diagnosis (Darweesh et al., 2016). Considering these factors and more, guidelines for the diagnosis of prodromal PD were published recently. The study applied their guidelines to existing data of patient cohorts and the assessment was shown to be 95% accurate in discerning prodromal PD. However, the guidelines have not yet been assessed for predictive accuracy in the general population (Mahlknecht et al., 2016).

PD presents in familial and idiopathic varieties. The vast majority of PD cases are idiopathic, but the familial connection indicates a genetic component to PD exists as well. Several genetic mutations have been found in conjunction with PD. These include mutations inherent in the genes encoding α -synuclein (SNCA), parkin (PRKN2) and superoxide dismutase-2 (SOD2), as well as other genes not covered in this paper

(DeMaagd & Philip, 2015; Miyazaki & Asanuma, 2008). These genetic mutations are salient markers of neurogenerative potential, but do not account for the pattern of degeneration observed in PD. Poulin et al. (2014) identified 96 genes that are potentially implicated in PD pathology regarding the selective vulnerability of SNc dopaminergic neurons. It is not yet clear which of these genes are responsible for familial PD (DeMaagd & Philip, 2015). While the genes mentioned here are shown to be involved in the pathology of PD, having a mutation in one gene does not guarantee that an individual will develop the disease (Mantero et al., 2017). Similarly, the ethics of genetic testing are often debated due to generalizability of data from specific patient populations, the predictive value of the results, and the financial cost of the test (DeMaagd & Philip, 2015). For these reasons, genetic testing cannot stand alone as a definitive diagnostic of PD.

Neuroimaging provides useful insight to the diagnosis and progression of PD. Magnetic resonance imaging (MRI) is a readily available, effective, and non-invasive technique for imaging the brain *in vivo*. Studies have found that the substantia nigra can be visualized by MRI, and have attempted to quantify its appearance on high (3T) and ultra-high (7T) field strength iron-sensitive MRI (Schwarz et al., 2014). The ventrolateral extensions of the SNc into the SNr, i.e. nigrosomes, are the most susceptible cell populations in PD pathology (Gibb & Lees, 1991). Nigrosome-1, located in the posterior third of the SN, was found to have a characteristic ‘swallow-tail’ shape in healthy study subjects. The swallow tail sign appears as a dark wedge on the 3T high field strength MRI constructed image. Researchers concluded that the presence of the swallow tail sign

on the 3T MRI was clearly indicative of the non-PD state, whereas absence of the swallow tail was associated with the PD state but was not conclusive. The swallow-tail could be absent due to factors, such as high iron content in the surrounding SN cells, that were not inherently indicative of PD (Schwarz et al., 2014).

Mahlknecht and colleagues (2017) emphasized the utility of high and ultra-high field strength MRI in their analysis of MRI techniques' accuracy at detecting PD. An MRI at regular field strength of a PD brain and that of a healthy brain appear the same for clinical purposes. Differences are only apparent at the high and ultra-high strength levels, as alluded to in the description of the swallow tail sign (Schwarz et al., 2014). The SNc of healthy patients is generally hypo-intense, with an ovoid shaped hyper-intensity along the dorsolateral border. This hyperintensity, called the dorsolateral nigral hyperintensity (DNH), is believed to correspond to nigrosome-1 based on correlation with a 7T post-mortem MRI study. The absence of DNH at least unilaterally in *in vivo* 3T MRI and a bilateral absence in an *in vivo* 7T image were found to be significant markers of PD pathology. Furthermore, the DNH was not absent in movement disorders that exhibit parkinsonism but do not follow the same pathology as PD. Absence of the DNH can potentially be a key diagnostic feature in the future (Mahlknecht et al., 2017).

There is a large body of research exploring the link between iron accumulation in the SN and PD pathology. One study purportedly established a correlation between iron-fortified diets early in life and developing PD later. Iron accumulates in the brain throughout life, and is mostly stored in glia, although some iron is stored in neurons. These pools continue to grow with age, until they are eventually overwhelmed. At this

point iron is released into the cytoplasm of neurons, where it interacts with dopamine and forms cytotoxic metabolites (Hare et al., 2017). This is corroborated in that higher than average iron levels are noted in the SNc in PD patient's brains (Tribl et al., 2009). Hare et al. (2017) found that incidence of PD after the age of 50 is higher in nations that adopted iron-fortification in dietary staples than in nations that have not mandated iron fortification. Additionally, incidence increased after these policies were implemented. It would be interesting to continue this line of research to determine any diagnostic significance of dietary iron intake, both in early life and during the disease (Hare et al., 2017).

DISCUSSION

Parkinson's Disease is a complex and multifaceted neurodegenerative disorder of dopamine in the midbrain, although many more systems and structures are involved beyond the basal ganglia. The ventral mesencephalon is perhaps best considered as the crux of the Parkinsonian model where all the diverse underlying processes converge, giving rise to the set of symptoms we recognize as Parkinson's Disease. Dopamine deficiency is the easily identifiable factor for causing the disease, but the mechanisms which cause reduced dopamine levels are numerous. It is impossible to say at this point if any single mechanism of degeneration is the quintessential 'parkinsonian' mechanism, since neuronal degeneration results from such a range of insults. What is likely, as alluded to by Poulin et al. (2014), is that PD arises from a multiple-hit model. Towards this end, it could be helpful to consider PD from a holistic perspective, as a dysregulation in the homeostatic interactions of multiple neurological systems.

Much research has been dedicated to explaining the selective susceptibility of neurons in the SNc. Vogt Weisenhorn et al (2016) provide an excellent account of the macro and micro-organization of the dopaminergic neurons in the ventral mesencephalon. The main macroscopic pathologic consequence in the ventral mesencephalon is the loss of the neurons that project from the SNc, primarily from the ventrolateral tier of the SNc to the striatum (Vogt Weisenhorn et al., 2016). This connection is not part of the three pathways commonly used to model connections in the BG circuit. Most models assume cortical inputs flow through the striatum or STN, then on to the Gpi or SNr. The nigrostriatal connection runs opposite the flow of information

in the direct or indirect pathway, likely as a feedback modulator (Yetnikoff et al., 2014). When dopamine is not present striatal activity increases pathologically. The resulting synchronization in BG circuits (Weinberger & Dostrovsky, 2011) is perhaps similar to a feedback between microphone and a speaker, where small input waves grow into chaotic output waves due to lack of dampening, although the physics of the BG and audio circuitry are admittedly vastly different. The conceptual BG circuit operates well with the view that dopamine is predominantly an inhibitory neurotransmitter within the BG. Still, the question of why the ventrolateral tier of the SNc degrades is not addressed.

The fact that multiple researchers have all found distinctions within the organization and biochemistry of the midbrain dopaminergic neurons indicates that heterogeneity within the region exists and is important to its function. From Dahlström and Fuxe (1964) until present, researchers continue to find spatial and molecular delineations within the midbrain. The cell groups A8-A10 align consistently with neuroanatomical structures, thus confirming that the organization of the brain follows both spatial and functional divisions. Since then, research has found overwhelming evidence for the differences and distinctions between the cell types, yet none of the characteristics can entirely explain the selective pathology observed in PD (Anderegg et al., 2015; Brichta & Greengard, 2014; Vogt Weisenhorn et al., 2016).

It seems that much of the literature is converging on the toxicity of oxidative stress as a major mechanism of cell death in PD. In this mechanism, oxidative stressors within the cell cause damage and death, which results in neuroinflammation that in turn causes more stress to cells (Crotty et al., 2017; Graumann et al., 2002). The susceptible

neurons in the SNc are characterized by a high baseline level of metabolic activity, with a resting metabolic state 3x more active than the neighboring VTA dopaminergic population (Vogt Weisenhorn et al., 2016). Discerning the cause or necessity of this high-energy state could be useful for early-stage therapies, before significant degeneration occurs.

The susceptibility of the small subset of dopaminergic neurons in the SNc could be explained by oxidative stressors overloading the compensatory mechanisms. Perhaps, in the VTA or other cell populations with more regular baseline metabolism, a surge in toxic oxidants could be quenched entirely by cellular mechanisms. There may be ample ALDH1a1 to enzymatically metabolize dopamine, dopamine levels could be kept in check before pathological amounts of quinones accrued, and neuromelanin would be able to handle the rest. In such a system, the cellular antioxidants, e.g. glutathione, would be able to handle the shock of an increased oxidative load (LaVoie & Hastings, 1999; Miyazaki & Asanuma, 2008; Sulzer & Zecca, 2000).

On the contrary, the SNc cells are already likely close to their capacity to handle oxidative loads. Larger quantities of oxidants build up over time, and a surge in the respiratory demands could theoretically push a cell past its threshold for repair. For example, glutathione is involved in a plethora of essential biochemical processes such as DNA repair and iron metabolism (Tribl et al., 2009). If high levels of glutathione have been covalently bonded to dopamine quinones, then the cell will experience damage from i) free radicals that glutathione cannot neutralize, and ii) the inability to perform necessary reactions to maintain cellular function and life (Crotty et al., 2017; Miyazaki &

Asanuma, 2008). There is also evidence to show that iron and other cations accumulate in the cells, further exacerbating cellular damage (Miyazaki & Asanuma, 2008; Tribl et al., 2009).

As cells in the SNc are predisposed to oxidative shock due to dense mitochondria in unmyelinated axons, and have high levels of potentially cytotoxic catecholamines (Rietdijk et al., 2017), it seems highly likely that the effects of oxidative stress are more prevalent in this population as compared to others. The account of molecular heterogeneity in dopaminergic subpopulations of the ventral mesencephalon confirm that the DA^{1A} subgroup lacked transcription factors conferring neuroprotection (Anderegg et al., 2015; Poulin et al., 2014; Vogt Weisenhorn et al., 2016), thus the molecular biology aligns well with the oxidative stress model. It would be fruitful if future research could continue to quantify this line of reasoning empirically.

Currently L-DOPA is the best therapy for treating PD, but it has some drawbacks (DeMaagd & Philip, 2015). As noted in Crotty et al. (2017) and others, increased levels of dopamine metabolites can have deleterious effects in a cell. Likewise, in the long term, L-DOPA therapy has been linked to dystonia and other neurological imbalances. For this reason, L-DOPA is often administered in conjunction with neuroprotective agents (DeMaagd & Philip, 2015).

An effective route for therapy is to block the α_2 adrenoceptor. This results in lower extracellular dopamine metabolites, which reduces the toxic threat to local neurons (Mejias-Aponte, 2016). There is a close interaction between the dopamine system and the adrenergic system, which opens up the adrenergic system as an organic tool by which to

control dopamine levels. Ideally, if the dopamine levels can be artificially controlled early in disease progression, then there is a possibility of delaying neuronal death. It is unfortunate that current treatment is only able to manage symptoms, and cannot yet slow disease progression (Breen & Lang, 2017).

The adrenergic system is also a potential candidate for the origin of neurotoxicity. In rodents, low levels of norepinephrine were associated with a low dopamine level, and induced parkinsonism. This effect was reversed by administering dopamine (Mejias-Aponte, 2016). If a lesion or imbalance occurred somewhere in the adrenergic system that led to decreased norepinephrine it would lower ambient dopamine levels, as in the rats. Likewise, it would be interesting to explore if an increase in norepinephrine levels could raise dopamine concentration to pathological levels and cause degeneration by oxidative stress, thus showing another possible origin of degeneration.

The protein α -synuclein is responsible for a portion of the pathology in PD as well. It has been implicated in Lewy body pathology and is indisputably an agent of degeneration in PD, Alzheimer's Disease, Huntington's Disease, and many other neurodegenerative diseases (DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016). Braak et al. (2003) treats α -synuclein as a prionic pathogen which enters the body in the nasal cavity and gut, then travels to the central nervous system along the vagus nerve. This system has been shown to reflect a majority of PD cases' progression, but it is not always accurate. The α -synuclein protein has reliably been shown to act as a prion by corrupting non-pathological α -synuclein proteins in a cell (Stuendl et al., 2016), and α -synuclein has been linked to cellular degradation (Vogt Weisenhorn et al., 2016).

However, the progression of α -synuclein pathology through the body, as measured by Lewy body deposition, does not always match the hypothesis of Braak et al. (2003) (Rietdijk et al., 2017). More studies are needed to determine the mechanism responsible for α -synuclein deposition that breaks from Braak's hypothesis.

Highly oxidative environments influence α -synuclein biology, favoring conversion of soluble α -synuclein to insoluble fibrils that oligomerize and aggregate. These aggregates form the Lewy bodies which are characteristic of the degeneration seen in PD and other neurodegenerative diseases (Crotty et al., 2017; Miyazaki & Asanuma, 2008). As noted, SNc dopaminergic neurons are highly susceptible to oxidative stress, and are therefore susceptible to α -synuclein induced degradation as well. This is another finding which gives insight as to why the SNc is especially vulnerable to degeneration.

Despite the efficiency of the oxidative stress model in explaining neurodegeneration in the SNc, there are still many inconsistencies. Degradation in the SNc does not progress at a uniform pace. PD is generally diagnosed when about 60% of dopaminergic neurons in the SNc have degraded (Vogt Weisenhorn et al., 2016). Kordower et al. (2013) found that at the earliest points post-diagnosis, 50-90% of the SNc dopaminergic neurons were degraded, and loss was virtually complete 4 years post-diagnosis. Worsening of symptoms after this point is likely due to loss of compensatory mechanisms. There is a large amount of research dedicated to ascertaining what causes that sudden and rapid decline in SNc neurons (Vogt Weisenhorn et al., 2016).

In terms of the multiple-hit hypothesis, it is possible that there is a balance of many symptoms maintaining the equilibrium of the BG, and several systems must be

damaged at once for pathogenesis to occur. High oxidative load is an attractive explanation for the pathology of PD, but it is essential to remember that the majority of the population does not develop PD in their lifetime. Incidence is around 1% of elderly adults in developed nations (Breen & Lang, 2017; Brichta & Greengard, 2014; DeMaagd & Philip, 2015; Vogt Weisenhorn et al., 2016). Even PD patients go many years without significant degeneration, before they develop symptoms. It is evident that high oxidative stress cannot alone be responsible for the PD pattern of neurodegeneration, otherwise all people would be prone to develop PD. Therefore is it possible that PD results from multiple mutations arising in critical genes within the systems that mitigate oxidative load, rendering the predisposed SNc cells more susceptible to oxidative stress, neuroinflammation, and neurodegeneration. A multiple-hit hypothesis as the etiology of PD accounts for the molecular and macroscopic pathology which gives rise to PD.

Since neurodegeneration occurs rapidly and early in disease progression, it would be helpful in terms of therapies to initiate treatment early. If a therapy could be developed to slow progression of the disease, it would be enormously beneficial to initiate treatment when SNc neurons are still alive. Even in current therapies, patients' quality of life benefits from early treatment (DeMaagd & Philip, 2015). Several potentially diagnostic markers have been identified on high power MRI images (Mahlknecht et al., 2017; Schwarz et al., 2014). Theoretically, if a patient has a history which aligns with criteria for prodromal diagnosis as per Mahlkecht et al. (2017), then they could receive routine diagnostic scans. This would ideally identify PD patients much earlier than the current

techniques, and maximize the result of therapies. There is a bright future for PD treatment, beginning with the diagnostics reviewed here.

CONCLUSION

PD is a complex neurodegenerative disease related to irregularities in dopamine biology in the ventral mesencephalon. Its formal study date back to the early 19th century when Dr. James Parkinson first described the motor symptoms we now know as parkinsonism. These motor symptoms are due to the depletion of dopaminergic neurons in the SN, which causes irregularities in neuronal firing patterns within the BG. Patients exhibiting the pathological firing patterns are unable to integrate smooth motion, and have significant cognitive deficits in tasks involving cortical executive function.

The degeneration of the SNc in PD pathology is complex, and tied to many brain systems. Dopaminergic neurons in the SNc degrade preferentially compared to neighboring dopaminergic populations in the VTA. It is proposed that this is due to higher baseline oxidative stress in the SNc, pathologic aggregation of α -synuclein, iron accumulation, and a variety of other factors. The dopaminergic system's interaction with other neurotransmitter systems opens the possibility of controlling dopamine levels by means other than exogenous L-DOPA, and are potential areas for future treatments.

Recent advances in neuroimaging present new opportunities for early detection of PD. Early detection of PD is essential to slowing disease progression, because more than 50% of the neurons in the SNc have degraded by the time clinicians make a diagnosis in the current system. L-DOPA therapy is the best therapy currently available. However, it is ultimately cytotoxic, so earlier treatment of the underlying cause of degeneration could

be more beneficial to the patients' quality of life as the disease progresses. Future research should focus on determining the reason that the SNc is especially susceptible, and determining the multi-hit mechanism of neurodegeneration. Clinicians and research currently focuses on maintaining patients' quality of life and management of symptoms as we progress towards the ultimate goal, a cure for PD.

Future Research

There is still a lot left to learn about PD. On a molecular level, it would be helpful to learn more about the differential expression of genes that play a role in conferring protection or susceptibility in the ventral mesencephalon dopaminergic complex. There is still no definitive consensus as to why the ventrolateral tier of the SNc degrades more than elsewhere in the SNc, and why the SNc is more susceptible than other dopaminergic neurons. It would also be interesting to explore the role of epigenetics in expression of the genes involved in these pathways, such as differential expression of transcription factors, and the differences in post-translational modifications of gene products in the SNc and VTA. Ideally, some of these differences could be diagnostically significant of PD and act as biomarkers to aid early diagnosis (Poulin et al., 2014).

The adrenergic system has a profound interaction with the dopaminergic system. Future research would be well spent to flesh out the details of this interface more completely. The adrenergic system offers an organic interface to manipulate dopamine levels *in-vivo*, without using exogenous dopamine metabolites that are known to be cytotoxic. Of course, a treatment such as this would need to be implemented early on in disease progression, so that there is still a significant quantity of dopaminergic neurons

extant in the SN when therapy is initiated. It is worth exploring the ability of the adrenergic inputs to the dopamine system to raise dopamine levels, since a lesion in the brain that increases norepinephrine expression could endogenously raise dopamine to pathological levels. There is also a limited affinity of norepinephrine for the dopamine receptors D2 and D4, so in the absence of dopamine it might be worthwhile to explore a possible compensatory role for norepinephrine (Mejias-Aponte, 2016).

Oxidative stress as a mechanism of degeneration is an area of ongoing research. Specifically, the fate of neuromelanin is not known. It is believed to confer neuroprotection on cells, but in the SNc it disappears. The disappearance of neuromelanin precedes cell death, as non-pigmented cells in the SNc of PD brains still express the tyrosine hydroxylase enzyme which regulates dopamine synthesis. It is also yet uncertain why neuromelanin is found in catecholaminergic cells containing dopamine and norepinephrine, but not in cells with epinephrine. Epinephrine is the only substitute amine out of the three, so it would be interesting to explore if secondary amines are unable to polymerize as primary amines can, and if this has any implications in the pathology of PD. Understanding the biology of neuromelanin and its metabolism could give insight as to a possible cure for oxidative stress-induced degeneration (Fedorow et al., 2005).

Mahlknecht et al. (2017) found that the absence of DNH was indicative of PD. Mahlknecht (2016) described symptoms to look for in patients who have not been diagnosed with PD, in order to diagnose them earlier, i.e. before the SN degrades so thoroughly. It would be helpful to combine the findings and create a statistical measure

that can assess a patient's symptoms and imaging results, and calculate an empirical diagnosis of PD. Of course, any such calculation would need to be verified by a clinician, but it could be very helpful especially in early diagnoses.

Researching the brain is complex. It is helpful to have models of brain function that can be used to manipulate the systems and structures that compose the brain, without inflicting invasive procedures on patients in the name of science. However, current technology is limited by processing power that falls far short of encompassing the intricacies of the brain. Technology clearly lags behind biology in this regard. Computational biologists would be wise to develop a model of the brain, or at least small parts of it, that can be manipulated. A tool like this would be quintessential in PD research (Helie et al., 2013).

PD is similar to several other degenerative diseases. More research is needed to discern the cause of PD, ALS, Alzheimer's Disease, and Lewy Body Dementia. Some researchers propose that these diseases share a common origin, and our classification of the disease depends on the pattern by which it spreads rather than by the underlying mechanism of the disease itself (Recabarren & Alarcón, 2017). A theory such as this, if proven true, would have unprecedented benefit for research of neurodegenerative diseases as a whole, and could drastically improve the way we conceptualize these diseases.

A final point of curiosity concerns the BG and their connections to nuclei not involved in PD pathology. The BG are connected to the limbic system via the ventral striatum, and they are implicated in many complex behaviors involving learning, and

reward pathways, such as addiction. It would be interesting to see if and how these behaviors change in a PD patient. This would give further insight into the nature of the connections of the BG and its role in cognition.

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CURRICULUM VITAE

