Université de Montréal

Theoretical And Experimental Considerations Of Selective Vulnerability In Parkinson's Disease

Par

Samuel Burke

Département de Neurosciences

Faculté de Médecine

Thèse présentée en vue de l'obtention du grade de Philosophiae Doctor (Ph.D. en neurosciences)

Novembre 2021

Université de Montréal

Département de Neurosciences

Faculté de Médecine

Cette thèse s'intitule:

Theoretical And Experimental Considerations Of Selective Vulnerability In Parkinson's Disease

Présenté par:

Samuel Burke

A été évaluée par un jury composé des personnes suivantes:

Michel Panisset
Président-rapporteur

Louis-Éric Trudeau
Directeur de recherche

Pierre Blanchet
Membre du jury

Birgit Liss
Examinateur externe


FIDE SPLENDET ET SCIENTIA

1 Résumé

Les maladies neurodégénératives sont typiquement caractérisées en fonction de leurs symptômes et des observations pathologiques effectuées après le décès. Les symptômes spécifiques à la maladie sont également normalement associés aux dysfonctionnements et à la dégénérescence de certaines sous-populations spécifiques de neurones dans le système nerveux. La maladie de Parkinson (MP) est une maladie neurodégénérative principalement caractérisée par des symptômes moteurs dus à la dégénérescence spécifique des neurones dopaminergiques (DA) de la *substantia nigra pars compacta (SNpc/SNc)*. Il semble cependant que les neurones DA de la SNc ne soient pas la seule population de neurones qui dégénère dans la MP. L'analyse post-mortem, l'imagerie *in vivo* et les symptômes cliniques démontrent que le dysfonctionnement et la dégénérescence se produisent dans plusieurs autres régions du système nerveux, incluant par exemple des neurones noradrénergiques (NA) du locus coeruleus (LC), des neurones sérotoninergiques des noyaux du raphé et des neurones cholinergiques du noyau moteur dorsal du nerf vague (DMV) et du noyau pédonculopontin.

Comme d'autres maladies neurodégénératives, la MP est causée par plusieurs facteurs, tant génétiques qu'environnementaux. De nombreuses observations suggèrent que ces facteurs soient associés au dysfonctionnement de plusieurs systèmes ou fonctions cellulaires incluant la production d'énergie par la mitochondrie, l'élimination de protéines dysfonctionnelles par le protéasome et le lysosome, la régulation de l'équilibre entre la production d'espèces oxydatives réactives et les mécanismes antioxydants, la régulation des niveaux de calcium intracellulaire et l'inflammation. Il semble donc que le dysfonctionnement de ces facteurs converge pour provoquer la dégénérescence neuronale dans le contexte du vieillissement. Ce qui rend les neurones de certaines régions du système nerveux intrinsèquement plus vulnérables aux facteurs associés à la MP est une question fondamentale qui n'est pas résolue pour le moment.

Les travaux de cette thèse sont basés sur l'hypothèse proposant que cette vulnérabilité sélective découle de propriétés communes retrouvées chez les neurones vulnérables. En particulier, les neurones vulnérables auraient en commun d'être des neurones de projections dotés d'un axone complexe qui projette sur de longues distances, formant un nombre très élevé de terminaisons axonales sur de vastes territoires du système nerveux. De plus, ces neurones présenteraient des propriétés physiologiques distinctives, incluant notamment une décharge autonome (pacemaker). Ensemble, ces caractéristiques pourraient contribuer à placer ces neurones dans des conditions de fonctionnement aux limites de leur capacités

bioénergétiques et homéostatiques, rendant difficile toute adaptation aux dysfonctionnements cellulaires associés au vieillissement et causés par les facteurs de risques de la MP.

Dans cette thèse, je présenterai une revue systématique de la littérature sur la perte de neurones dans le cerveau des personnes atteintes de la maladie de Parkinson, montrant que l'identité neurochimique précise des neurones qui dégénèrent dans la maladie de Parkinson, et l'ordre temporel dans lequel cela se produit, n'est pas clair. Cependant, en analysant les points de vue présentés dans les publications citant cette revue, nous avons remarqué que la majorité de ceux-ci ne reflètent pas le message central de notre publication. Puisque l'identification de l'identité des neurones vulnérables et non vulnérables à la MP est fondamentale pour le développement de théories et hypothèses sur les causes de la MP, nous suivons cette première publication avec une lettre réaffirmant l'importance de faire face aux problèmes identifiés dans notre revue.

Nous présentons ensuite des données *in vitro* montrant que les neurones vulnérables à la MP, comparés à ceux qui sont moins vulnérables, ont une capacité intrinsèque à développer des champs axonaux plus importants et plus complexes, avec un nombre plus élevé de sites actifs de libération de neurotransmetteurs. De plus, nous constatons que ces observations sont corrélées à une vulnérabilité plus élevée face à un stress oxydatif pertinent pour la MP. Ces données sont en accord avec l'hypothèse selon laquelle le domaine axonal, et en particulier le nombre de sites de libération de neurotransmetteurs par neurone, est un facteur important qui contribue à rendre un neurone sélectivement vulnérable dans le contexte de la MP.

Enfin, nous présentons une méthode d'analyse d'image *open-source* visant à aider les biologistes et les neuroscientifiques à automatiser la quantification du nombre de neurones dans des cultures primaires de neurones, telle qu'utilisée dans les travaux de cette thèse. Nous proposons que cet algorithme simple — mais robuste — permettra aux biologistes d'automatiser le comptage des neurones avec une grande précision, quelque chose de difficile à effectuer avec les autres approches *open-source* disponibles présentement.

Nous espérons que les travaux présentés dans cette thèse permettront de contribuer à raffiner les théories visant à expliquer l'origine de la MP et à terme, de développer de nouvelles approches thérapeutiques.

Mots-clés: Maladie de Parkinson, vulnérabilité sélective, neurodégénérescence, arborisation axonale

2 Abstract

Neurodegenerative diseases are typically characterized based on their symptoms, and pathological factors identified after death. The disease-specific symptoms are due to the dysfunction and degeneration of specific subpopulations of neurons, which cause dysfunction in particular brain functions. Parkinson's disease (PD) is a neurodegenerative disease primarily characterized by motor symptoms due to the specific degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNpc/SNc): a population of neurons essential for motor control. SNc DA neurons are, however, not the only population of neurons that degenerate in PD. Post-mortem analysis, *in vivo* imaging, and clinical symptoms demonstrate that dysfunction and degeneration occur in several other neuronal nuclei. These include, but are not limited to, noradrenergic (NA) locus coeruleus (LC) neurons, serotonin neurons of the raphe nuclei, and cholinergic neurons of the dorsal motor nucleus of the vagus (DMV) and pedunculopontine nucleus.

Like other neurodegenerative diseases, PD is linked to several risk factors, both genetic and environmental. The evidence suggests that these risk factors are associated with the dysfunction in systems of cellular bioenergetics (including mitochondrial function); proteostatic homeostasis; endolysosomal function; an imbalance between the production of reactive oxidative species (ROS), and antioxidant mechanisms; calcium homeostasis; alpha-synuclein misfolding; and neuroinflammation. Consequently, together with aging, these risk factors converge on causing the selective degeneration of "PD-vulnerable" nuclei. What makes these neurons intrinsically vulnerable to PD-associated risk factors is a fundamental question — and understanding *these neurons* will reveal biological mechanisms that we can target to protect these cells from degeneration.

Our best hypotheses to explain *why these neurons* are based on the observations that most PD-vulnerable neurons are long-range projection neuromodulatory neurons sharing common characteristics: projecting to voluminous territories, having very long and highly branched unmyelinated axonal domains with vast numbers of neurotransmitter release sites, and exhibiting a unique physiology such as pacemaker firing. Taken together, this suggests that these neurons function at the tail-end of their bioenergetic and homeostatic capacity, unable to tolerate any further demands, such as those incurred in the presence of risk factors associated with PD.

In this thesis, I will present a systematic review on the literature on purported cell loss in PD brains, showing that — given the actual primary evidence — the precise neurochemical identity of neurons that degenerate in PD, and the temporal order of this degeneration, is far less clear than described by most publications. This review — at the time of writing — has gone on to be highly cited. However, analyzing

the claims made in publications citing this review, we discover that the majority of claims do not reflect the core message of our publication. Since the identity of PD-vulnerable and non-PD-vulnerable neurons is fundamental to theory and hypotheses when trying to understand PD, we follow this first publication with a letter restating the importance to address our observations.

We then present *in vitro* data showing that classically PD-vulnerable neurons, when compared to non-PD vulnerable neurons, have an intrinsic capacity to develop larger and more complex axonal domains, with higher numbers of active neurotransmitter release sites. Moreover, we find that these observations correlate to elevated vulnerability to PD-relevant stress assays. These data provide additional support for the hypothesis that the axonal domain — and in particular — the number of active neurotransmitter sites per neuron, is a cell-autonomous factor rendering a neuron selectively vulnerable in the context of PD.

Finally, we present an open-source tool to support biologists and neuroscientists in automating the quantification of neuron numbers in medium-throughput primary cell cultures. Where the application of other open-source solutions is either too simplistic for the use-case or technically challenging to implement, this simple — yet robust algorithm — allows biologists with limited computational nous to automate neuron counting with high precision.

We hope that the work presented in this thesis will contribute to the refinement of theories aimed at explaining the origin of PD and, ultimately, to the development of new therapeutic approaches.

Keywords: Parkinson's disease, selective vulnerability, neurodegeneration, axonal arborization

3 Contents

4 List of tables

5 List of figures

6 Acronyms and abbreviations

Français

DA: dopaminergiques

DMV: noyau moteur dorsal du nerf vague

MP: maladie de Parkinson

NA: noradrénergiques

English

6-OHDA: 6-hydroxydopamine

AADC: aromatic amino acid decarboxylase

AD: Alzheimer's disease

ALS: Amyotrophic lateral sclerosis

ATP: Adenosine triphosphate

Aβ: amyloid precursor protein

BBB: Blood brain barrier

CNS: Central nervous system

COMT: catechol-Omethyltransferase

D1R: Dopamine D1 receptor

D2R: Dopamine D2 receptor

DA: dopamine

DBS: Deep brain stimulation

DLB: Dementia with Lewy Bodies

DMV: dorsal motor nucleus of the vagus

DOPAC: 3,4-dioxy-phenylacetic acid

ENS: Enteric nervous system

GWAS: genome-wide association study

L-DOPA: levodopa and l-3,4-dihydroxyphenylalanine

LC: locus coeruleus

LP: Lewy pathology

MAOB: Monoamine oxidase type B

MMP+: N-methyl-4-phenylpyridine

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MSA: Multiple systems atrophy

NBM: Nucleus basalis of Meynert

OB: Olfactory bulb

PD: Parkinson's/Parkinson disease

PNS: Peripheral nervous system

PPN: Pedunculopontine nucleus

RBD: REM sleep behaviour disorder

REM: Rapid eye movement

RN: Raphe nuclei

ROS: reactive oxidative species

SNc/SNpc: substantia nigra pars compacta

STEM: Science, technology, engineering, and mathematics

STN: Subthalmic nucleus

TDP-43: transactivation response DNA binding protein 43

TH: Tyrosine hydroxylase

7 Overture

Life hangs on a very thin thread and the cancer of time is complacency. If you are going to do something, do it now. Tomorrow is too late. (Pete Goss, 1998)

This quote provides insight that I hope to have learnt, that I hope to live by, and that I hope to see embodied by the zeitgeist of the scientific community. I have been fortunate to be in good health — for many this is not the case, and scientific progress is the only hope. Would I be here had penicillin been discovered merely a handful of years later? I suspect not. How many more people would have suffered and died had this discovery arrived later; had the wheels of discovery been rotating slower, less efficiently, and with incentives even less aligned with the actual point of science, to understand and discover, let alone in the midst of WWII? This thesis is not about the scientific method, process, nor philosophy of science. However, these ideas have been the most important and enlightening insights I believe to have gained during this PhD. My hope for the future is that we can continue to improve our scientific process, scientific training, the management and drive to deploy capital to make this world a better place for generations to come.

To those waiting for a cure, present and future

8 Acknowledgements

First and foremost, I would like to extend my immense gratitude to my supervisor Louis-Eric Trudeau. Thank you for giving me the opportunity to explore. Every doctoral student is different, and you have extended your mentorship to allow me the freedom and opportunity to pursue tackling the questions lain out before me when embarking on this PhD; despite the number of dead-ends I went down. I am very grateful for the number of high calibre international conferences and summer schools you have enabled me to attend; some of the most enjoyable and interesting experiences of my scientific career. Finally, I am immensely grateful for the entertainment of my existential questions on the philosophy of science, and the process of science — often at the cost of actually doing science (counting cells!) — allowing me to discover my own path, values, and future. I would also like to thank Ed Ruthazer for his support in many an application, as well as availability and friendship at numerous conferences: I am sure that without your support I would not have managed to nag an FRQS doctoral training award! Thank you Borhane Annabi for giving me my first opportunity in science in Canada and helping me to navigate the wild west of academia strategically! Michel Cayouette and Frederic Charron, thank you for your participation in my *comité de parrainage.* I thoroughly enjoyed our discussions. Your guidance during my thesis helped steer us in a more fruitful direction as well as helping me get to the stage of wrapping this all up!

To members of the Trudeau lab, thank you for your friendship, coffees, and discussions: Benoît Delignat-Lavaud, Charles Ducrot, Ding Yuan Guo, Willemieke Kouwenhoven, Jana Kano, Natascha Klaus, Noémie Bélanger, Dominic Falardeau, Aurélie Fallon, and Nicolas Giguère. Also, thank you to my interns — my guineapigs for a first attempt at supervision and management — I am sorry for my own learning curve! Thank you so much to David Kysela for all your help with imaging and image analysis. Thank you sincerely to all the organizations that have supported me financially — firstly, Louis-Eric Trudeau, making the funds available from his grants, and then to the following organizations and prizes: The FRQS, the Faculty of Medicine, the Department of Neuroscience, the Prix d'excellence Bourse Solange-Saul and the Prix d'excellence Bourse Gertrude Poupart. This support providing me both the intellectual autonomy to pursue the questions in this thesis, as well as a pride and validation for my efforts.

I would like to thank Benjamin Stecher for his inspiring work, exchanges at conferences, nights out, and dinners. Amy Bernard for your wonderful conversation at multiple conferences and acceptance to come and talk at Neurosymposium. Thank you to all those involved in both the Neurophotonics summer school and the Neuromatch Academy. Thank you, Jim Surmeier, for your discussions at the GRC and for the viral particles than enabled a whole new aspect of my project (that worked — almost — first time).

All acknowledgement pages of a Trudeau Lab thesis/memoire necessitate a special section dedicated to Marie-Josée Bourque. Thank you. I cherish the friendship we have developed and hope to nurture it in the years to come. I admire and respect immensely the care and time you have sacrificed and dedicated to all the young scientists who come up and interrupt your actual work. I can categorically claim that I have not come across such devotion to the wellbeing and success of others in any other walk of life.

To my family, my mother, Giggia, and father, Johnny, thank you for asking questions, for being interested, for allowing me to make it here — encouraging me to jump at opportunity and go on an adventure. Buzz and Morghy, I love you.

Ma chère Noem, mon cher Edouard. There is life before, and life after. I started this marathon before — and if this is what life had to put me through for this outcome — it has been worth it. Serendipity has gifted me this path which I love and am excited for in a way I could not have imagined. Noem, thank you for inspiring me to try to emulate you. To get things done, to focus; to be my antithesis. Je t'aime

9 Publications and manuscripts

9.01 Primary

1. Burke Nanni, S., Giguère, N., & Trudeau, L.-E. (2018). On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease. Frontiers in Neurology, 9, 455. https://doi.org/10.3389/fneur.2018.00455
2. Burke, S., Trudeau, LE. (2021). On cell loss in Parkinson's disease, and the citations that followed. npj Parkinson's disease.
3. Burke, S., Bourque, MJ., Trudeau, LE. (2022). Axonal domain structure and function as a key cell-autonomous characteristic of selective vulnerability in Parkinson's disease. [accepted eNeuro]
4. Burke, S., Tchung, Alex., Trudeau, LE. (2021). Simple and reproducible methods for counting primary projecting neurons in vitro. [in preparation]

9.02 Collaborations

5. *Lunt, W., Burke, S., et al. Neurochemical identity and anatomy of cell loss in Parkinson's disease, and the clinical data available for correlation of progression: a systematic review and meta-analysis. PROSPERO 2021 (2021). *(co-first author)
6. Delignat-Lavaud, B., Kano, J., Ducrot, C., Massé, I., Mukherjee, S., Giguère, N., Moquin, L., Lévesque, C., Nanni, S. B., Bourque, M.-J., Rosa-Neto, P., Lévesque, D., Beaumont, L. D., & Trudeau, L.-É. (2021). The calcium sensor synaptotagmin-1 is critical for phasic axonal dopamine release in the striatum and mesencephalon, but is dispensable for basic motor behaviors in mice. BioRxiv, 2021.09.15.460511. https://doi.org/10.1101/2021.09.15.460511
7. Ducrot, C., Carvalho, G. de, Delignat-Lavaud, B., Delmas, C., Giguere, N., Mukherjee, S., Burke, S., Bourque, M.-J., Parent, M., Chen, L. Y., & Trudeau, L.-E. (2021). Neurexins Regulate GABA Co-release by Dopamine Neurons. BioRxiv, 2021.10.17.464666. https://doi.org/10.1101/2021.10.17.464666
8. Ducrot, C., Bourque, M.-J., Delmas, C. V. L., Racine, A.-S., Guadarrama Bello, D., Delignat-Lavaud, B., Domenic Lycas, M., Fallon, A., Michaud-Tardif, C., Burke Nanni, S., Herborg, F., Gether, U., Nanci, A., Takahashi, H., Parent, M., & Trudeau, L.-E. (2021). Dopaminergic neurons establish a distinctive axonal arbor with a majority of non-synaptic terminals. The FASEB Journal, 35(8), e21791. https://doi.org/10/gmz763
9. Wong, Y. C., Luk, K., Purtell, K., Burke Nanni, S., Stoessl, A. J., Trudeau, L., Yue, Z., Krainc, D., Oertel, W., Obeso, J. A., & Volpicelli-Daley, L. A. (2019). Neuronal vulnerability in Parkinson disease: Should the focus be on axons and synaptic terminals? Movement Disorders, 34(10), 1406–1422. https://doi.org/10.1002/mds.27823

10 Chapter 1 — Introduction

10.01 Aims

In this introduction, our current understanding of Parkinson's disease (PD) aetiology is described, the principles of selective vulnerability (within the context of PD), and our models to understand why PD affects the neurons that it does.

10.02 Neurological disorders

According to the World Health Organization, brain disorders represent a significant burden to all nations (low-, middle-, and high-income) (*Brain Health*, 2021). In 2017, globally, neurological disorders were the 7th most significant cause of disease burden, accounting for ~ 4% of total disease burden estimated by disability-adjusted life years (up from 2.7% in 1990) (Roser & Ritchie, 2016) — see Figure 1.

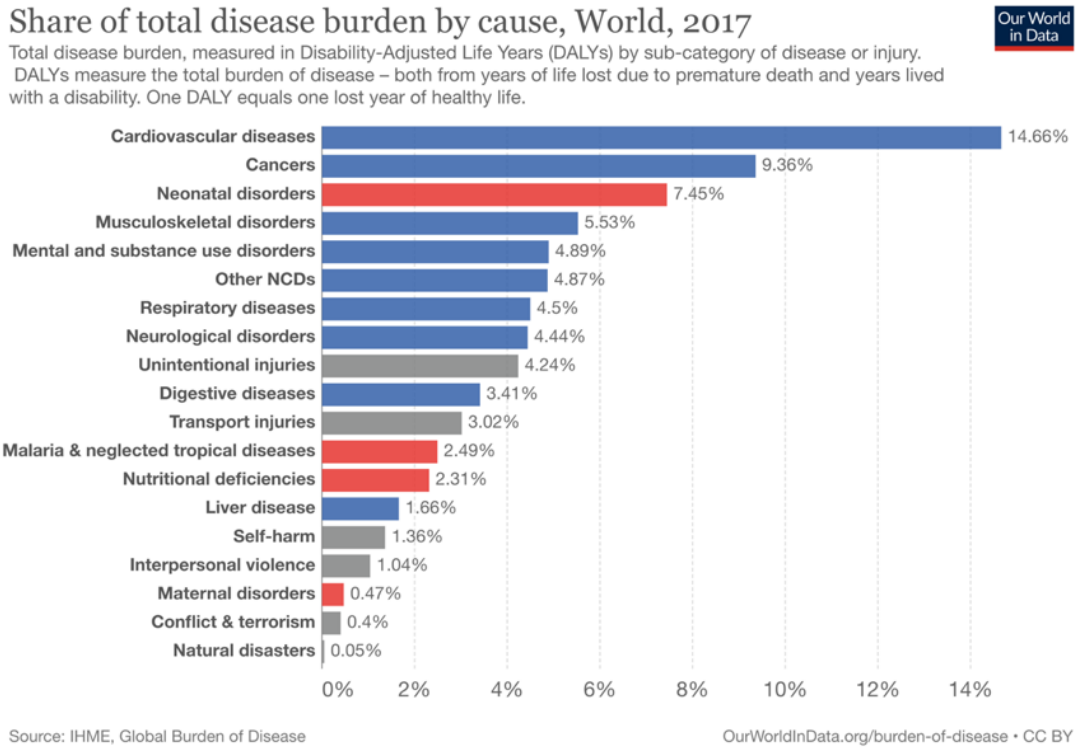


Figure 1 Global disease burden in 2017

(Roser & Ritchie, 2016)

10.03 Neurodegenerative diseases — global impacts

Neurodegenerative diseases are a subset of neurological disorders characterized by neurodegeneration: dysfunction and death of the brain parenchyma, resulting in significant deficits in normal brain function. ("Neurodegeneration," 2021). The leading risk factor for most neurodegenerative diseases is age (Hou et al., 2019). According to the United Nations 2019 report on World Population Ageing, "older people account for more than one fifth of the population in 17 countries today, and the United Nations Department of Economic and Social Affairs Population Division's projections to the end of the century indicate that this will be the case in 2100 for 155 countries, covering a majority (61 per cent) of the world's population" (United Nations et al., 2020). Thus, given our ever-ageing global population, if we are to avoid the catastrophic consequences of generations afflicted by neurodegenerative disorders, progress in the understanding the causes of neurodegenerative diseases is essential.

10.04 Neurodegenerative diseases

Neurodegenerative diseases are classified by the symptoms they cause, and the subsequent neuropathological findings that are found in the central or peripheral nervous system of the people affected by these diseases. Broadly, these symptoms involve either disorders of voluntary and involuntary movement, or cognitive function; followed, most often, by subsequent neuropathological findings of protein accumulation in restricted neuroanatomically defined regions. The major groups of neurodegenerative diseases fall under the umbrellas of: amyloidoses, tauopathies, α-synucleinopathies, and transactivation response DNA binding protein 43 (TDP-43) proteinopathies (Dugger & Dickson, 2017).

Amyloidoses are diseases where there is an accumulation of filamentous aggregated protein — amyloid — forming its characteristic β-sheet-rich secondary structure from protein monomers that have a propensity to fibrilise. The most common of these, Alzheimer's Disease, is associated with amyloid of amyloid precursor protein (Aβ). Other amyloidoses include Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker disease, and familial British and Danish dementias. Alzheimer's disease is, however, a mixed proteinopathy that also exhibits tauopathy: pathological accumulation of tau protein in neurons and glia. Other tauopathies include Progressive Supranuclear Palsy, Corticobasal Degeneration, Argyrophilic Grain Disease, and Pick's Disease (Dugger & Dickson, 2017; Knopman et al., 2021).

Synucleopathies are a class of neurodegenerative diseases characterized by the accumulation of the pre-synaptic protein, α-synuclein. α-synuclein is the major constituent of Lewy Bodies, neuronal inclusions — whose presence on neuropathological exam — give rise to the diagnosis of Lewy Body Disorders such as Parkinson's Disease (as well as Parkinson's Disease with Dementia, and Dementia with Lewy Bodies).

Other synucleopoathies include Multiple System Atrophy. TDP-43 proteinopathies include Amyotrophic Lateral Sclerosis, where the major component of neuronal inclusions includes the protein TDP-43. TDP-43 can also be found enriched in neuronal inclusions occurring in Frontotemporal Lobar Degeneration (Dugger & Dickson, 2017; Poewe et al., 2017a).

Of the major neurodegenerative diseases, Huntington's Disease and Multiple Sclerosis do not show canonical neuropathological findings of protein-rich inclusions, associated with most other neurodegenerative diseases. Huntington's Disease is the most common mono-genetic neuronal disorder, and Multiple Sclerosis a highly heterogenous chronic inflammatory disorder (Bates et al., 2015; Filippi, 2018), and are both characterized by restricted neuronal atrophy.

In all, though often presented as distinct entities, these diseases share many commonalities such as the neuropathological findings, protein abnormalities, and clinical symptoms, with significant overlap (Dugger & Dickson, 2017).

10.05 Parkinson's Disease

Parkinson's Disease (PD) is the fastest growing neurological disorder, and as of 2016, around 6.1 million people were living with PD globally — more than double than in 1990: a change mainly attributed to an ageing population (Dorsey et al., 2018). These figures are of course estimates: seminal work in 2007 (Dorsey et al., 2007) made predictions that, globally, in 2030 there would be ~9 million people diagnosed with PD. However, further work on estimating current and future prevalence suggests that this is an underestimate. Marras et al., (2018) suggest that by 2030, in the US, we can expect 1.23 million people to be living with PD (compared to 0.61 million estimated by Dorsey et al.), a difference of almost two-fold.

There is of course the evident tragedy a disease such as PD entails for the individual, but also an extreme economic burden associated with this disease. In the USA, for example, the economic burden of PD has been estimated to be worth around $52 billion annually, and research spending by the NIH on PD-specific work is around $160M annually (only 6.5% of the total spend on neurodegenerative diseases — private institutions such as the Michael J Fox foundation, add to this amount (around $100M annually)) (Schekman & Riley, 2019).

10.06A (brief) history

> "So slight and nearly imperceptible are the first inroads of this malady, and so extremely slow its progress, that it rarely happens, that the patient can form any recollection of the precise period of its commencement" — James Parkinson, 1817

PD was first described as a neurological disorder — within our modern literature — in 1817 by James Parkinson (Parkinson, 1817). Descriptions however, of a PD-like disorder, can be found in older western medical texts between the 1600s and 1800s as well as in traditional Indian and Chinese texts from 1000 BC (Goetz, 2011; Stern, 1989; Zhang et al., 2006).

Following the classical essay by James Parkinson in 1817, PD was further described by Jean-Martin Charcot (coining the term *PD*, in 1872), where the distinction of cardinal motor symptoms began. Charcot's work led to the distinction of PD from other disorders of the central nervous system, such as multiple sclerosis, and led to the identification of sub-types of PD: with and without tremor, with extended posture, and with hemiplegia. Fritz Heinrich Lewy describes inclusions located outside the substantia nigra in 1912, followed by the discovery of inclusions within the substantia nigra by Konstantin Tretiakoff in 1919 (Trétiakoff, 1919), naming them after Lewy. The first pharmacological treatments for PD were belladonna alkaloids, agents that (discovered after the fact) have an anticholinergic mechanism of action. It was only once the discovery of the dopamine system became more concrete, in the 1950-60s — dopamine having been first synthesized in 1910 by Barger and Ewens — that it was discovered that there was an important relationship between dopamine, the striatum, and the control of motor output (Hornykiewicz, 2002 — a fantastic historical lecture on dopamine). The first evidence of robust therapeutic utility of levodopa (dopamine replacement therapy) was published by Birkmayer & Hornykiewicz (1961). The development of L-DOPA therapy was also pioneered in Montreal by André Barbeau, where the use and utility of L-DOPA as a therapy was optimized (Barbeau, 1969). The use of L-DOPA subsequently led to the use of dopamine agonists and inhibitors (of dopamine metabolism) to increase bioavailability (Goedert et al., 2013; Goetz, 2011). Further key and notable discoveries are listed in Table 1.

Year	Observations and discoveries	Notable individuals
2000–425 BC	Brief descriptions of PD-like symptoms appear in many ancient books (Ancient China)	
169 AD	First recorded distinction between resting and intentional tremor	Galen
1228	Description of a possible case of PD in China	Zihe Zhang
1584	Described tremors that involve hands, feet, and head	Yikvi Sun
1690	Described similar clinical symptoms to that of modern-day PD	Ferenc Papai Pariz
1817	Publishes “An essay on the shaking palsy”	James Parkinson
1872	“PD” is given the name — Parkinson’s Disease	Jean-Martin Charcot
1893	Described parkinsonian tremor in a patient with a tumour in the substantia nigra	Paul Blocq and Georges Marinesco
1899	Suggestion that the SNc could be the site of PD pathology	Édouard Brissaud
1912–1919	“Inclusions” located in SNc in PD, described	Frederick Heinrich Lewy and Konstantin Tretiakoff
1917	Encephalitis lethargica is described	Constantin von Economo
1919	Describes inclusions in the substantia nigra in PD and names them after Lewy	Konstantin Tretiakoff
1920	Propose that the anatomically distinct striatal system is also chemically distinct	Cécile and Oscar Vogt
1938	Confirmation that degeneration of the pars compacta of the substantia nigra causes parkinsonism	R Hassler
1940	First neurosurgical intervention of the basal ganglia to treat PD	
1957	Reserpine is reported to reduce motor activity in animals, which was reversed by L-DOPA	
1958	Demonstration of dopamine in the brain by histochemical methods	
1960	Found dopamine levels reduced in the striatum of PD	Oleh Hornykiewicz
1961	L-dopa as the first effective anti-PD drug and reports of the ALS–PD–dementia complex of Guam	
1962	Demonstration that low oral doses of L-DOPA in humans have anti-PD effects	
1965	Demonstration that mechanical lesions of the striatum cause a loss of dopamine in the substantia nigra and vice versa	
1967	Hoehn-Yahr scale for staging PD progression	
1970	Introduced DBS for treating the tremor of PD	Alim-Louis Benabid

1973	Publishes “Awakenings”	Oliver Sacks
1975	Hyposmia is recognized as a nonmotor symptom of PD	
1983	Report of a group of drug users who developed acute parkinsonism after MPTP exposure: MPTP induced parkinsonism	
1984	Lewy pathology found in enteric nervous system in PD	
1986	Description of rapid eye movement sleep behaviour disorder	
1987	Tested antiparkinsonian effect of apomorphine	George Cotzias
1988	Demonstration that pigmented neurons in the substantia nigra are particularly vulnerable to degeneration in PD and first description of microgliosis in the substantia nigra in PD	
1989	Introduction of the direct and indirect pathway model of the basal ganglia circuitry	
1990	Reversal of experimental parkinsonism by lesions of the subthalamic nucleus and complex I deficit detected in PD brains	
1995	Deep brain stimulation of the subthalamic nucleus becomes effective for the treatment of PD	
1997	Mutations in SNCA identified as the first genetic causes of PD	
1998	α-Synuclein found to be the main component of Lewy bodies	
2001	First double-blind controlled trial of a cell-based therapy in PD	
2003	Publish Braak staging	Kelly Del Tredici , Eva Braak, and Heiko Braak
2003	Multiplication of SNCA found to cause PD	
2003–2006	Identification of DJ1, PINK1, NR4A2, LRRK2, GBA, and ATP13A2	
2008	First suggestion of cell-to-cell transmission of α-synuclein	
2008–2010	Parkin–PINK1 reported to regulate mitophagy	
2012	First phase I clinical trial for immunotherapy in PD	
2014	Hypotheses on α-synuclein cell-to-cell transmission proliferate	

Table 1 Notable discoveries

(Goedert et al., 2013; Li & Le, 2017; Przedborski, 2017)

10.07 Epidemiology

Five to > 35 new cases of PD are diagnosed yearly, per 100,000 people, and a diagnosis is rare before the age of 50. Furthermore, as with many neurological disorders, prevalence increases by five to 10-fold between the ages of 60 and 90. Intriguingly, in most populations, incidence in men can reach roughly double that of women. However, causal factors have been difficult to isolate. Incidence rates are also found to be higher in individuals exposed to pesticides and traumatic brain injury. Incidence in sub-populations (such as those defined by race, ethnicity, genotype, and/or environment) have been reported to vary, however, these are inconclusive and it has been difficult differentiate between environmental interactions (Poewe et al., 2017a).

10.08 Symptoms and diagnosis

PD is a neurodegenerative disorder characterised by both motor and non-motor symptoms. Given the complexity of neurological disorders, it is difficult to attain specificity in their categorization, an element of variance that is very difficult to understand and accommodate for. Since the first description by James Parkinson over 200 years ago (Parkinson, 1817), a diagnosis of PD has varied in terms of its clinical definition. Figure 2 depicts the major symptoms associated with PD (tremor, rigidity, bradykinesia, dystonia, gait issues, as well as host of autonomic, psychiatric, and cognitive symptoms).

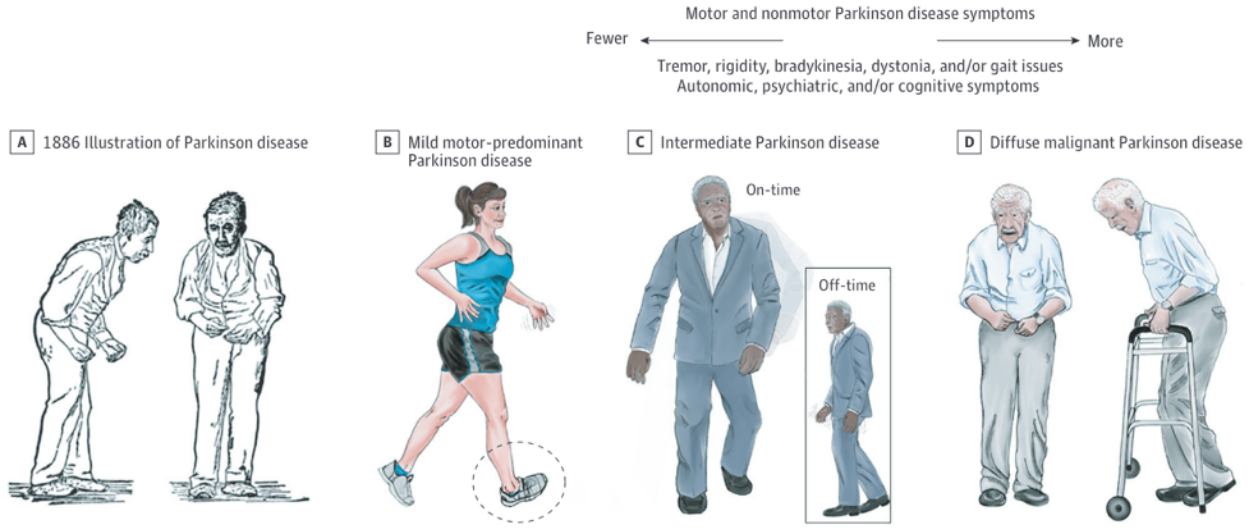


Figure 2 Parkinson's disease — summarizing a complex clinical phenotype

Panel **A** shows the canonical sketch drawn by William Gowers, in 1886 (Gowers, 1886). Panels **B** through **D** depict a more modern appreciation for the progressive nature and appearance of PD symptoms: going from early — mild and mainly motor manifestations — progressing to intermediate and late disease, where more systemic and broad symptoms arise (Armstrong & Okun, 2020b).

As of 2015, our most current definition involves a broad range of criteria (Table 2) (Postuma et al., 2015). For these renewed criteria, three years following publication, the same authors reported "high sensitivity and specificity compared with the gold standard" (Postuma et al., 2018).

Step 1	Diagnosis of parkinsonism (core feature)
	• Presence of bradykinesia as a slowness of movement and a decrement in amplitude or speed (or progressive hesitations or halts) as movements are continued In combination with at least one of: • rigidity and/or rest tremor
Step 2	Determining Parkinson disease as the cause of parkinsonism with two levels of diagnostic certainty
	Diagnosis of clinically established Parkinson disease requires all three of the below parameters: • Absence of absolute exclusion criteria. These criteria include clinical or imaging evidence for alternate diagnoses of parkinsonism, such as atypical parkinsonism, drug-induced parkinsonism or essential tremor. • Two or more supportive criteria. These include L-DOPA responsiveness, the presence of classic rest tremor, the presence of L-DOPA-induced dyskinesias, the presence of either olfactory loss or cardiac sympathetic denervation on metaiodobenzylguanidine (MIBG) scintigraphy. • No red flags This refers to features that are unusual but not absolutely exclusionary for Parkinson disease, for example, the rapid progression of gait impairment that requires wheelchair use or the development of severe autonomic failure within 5 years after onset.

Table 2 MDS Clinical Diagnostic Criteria for Parkinson's Disease

Summary adapted from (Postuma et al., 2015)

An important aspect of these revised criteria includes the consideration for non-motor features — bringing attention to the long prodromal period found in PD (Figure 3). Prior to the onset of motor symptoms, a person who will go on to develop PD, will likely begin exhibiting non-motor symptoms such as REM sleep behaviour disorder (RBD), depression, constipation, anxiety, and/or hyposomia. The onset of motor-symptoms may often concur with excessive daytime sleepiness prior to the noticeable onset of bradykinesia, rigidity, and tremor. During early-stage PD — where motor symptoms are often well controlled with current therapies — the onset of other non-motor symptoms such as fatigue, pain (Blanchet & Brefel-Courbon, 2018), mild cognitive impairment, and apathy, may occur. As the disease progresses from mid-stage to late-stage PD, motor symptoms stop responding well to treatment, resulting in increased fluctuations and dyskinesias. Concurrently, the individual may begin having urinary symptoms and

orthostatic hypotension, and eventually psychotic symptoms and/or dementia. Towards late-stage disease — prior to the need for institutionalization — the individual will likely experience axial deformities, dysphagia, increased postural instability, and gait disorders, followed by falls (Kalia & Lang, 2015; Poewe et al., 2017b). This short description, does however, little service to the diversity of *PDs* experienced by patients. This is reflected in the attempts that have been made to categories PD-subtypes: whether by genetic background or clinical phenotype, there is no consensus on how to give a finer categorization of all the different diseases experienced by those diagnosed with PD (Marras & Lang, 2013).

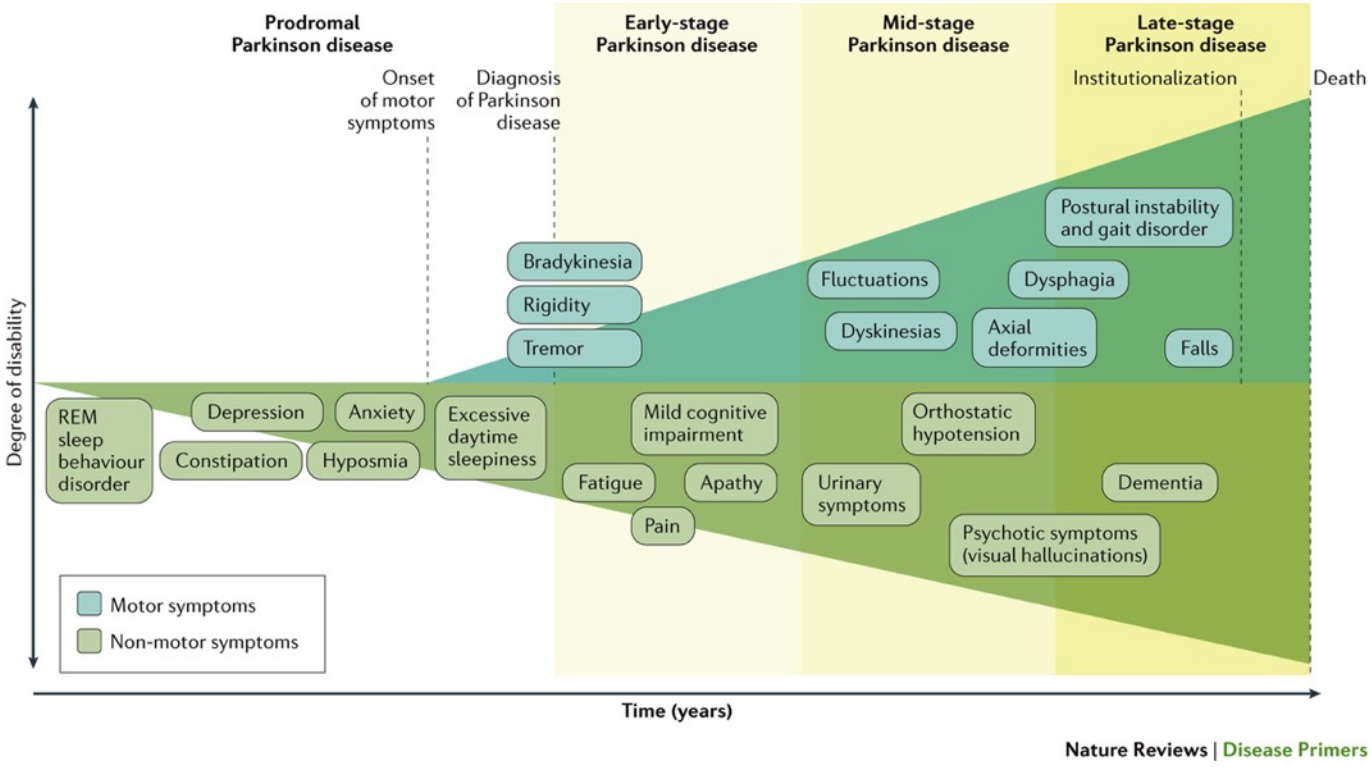


Figure 3 Symptoms in progression of PD

(Poewe et al., 2017b)

An increasingly interesting and informative observation is the association between RBD (rapid eye movement (REM) sleep behaviour disorder) and synucleopathies (including PD) (Postuma et al., 2012). Today, RBD is considered as a manifestation of α-synuclein pathology, with almost all individuals diagnosed with RBD converting to some form of synucleopathy within 15 years of diagnosis (Figure 4). This observation gives perspective into the prodromal phase of these disorders, but also revealing the dysfunction of systems that are not simply part of the basal ganglia (PD often being understood primarily as a dysfunction of this system, and as a motor-disorder).

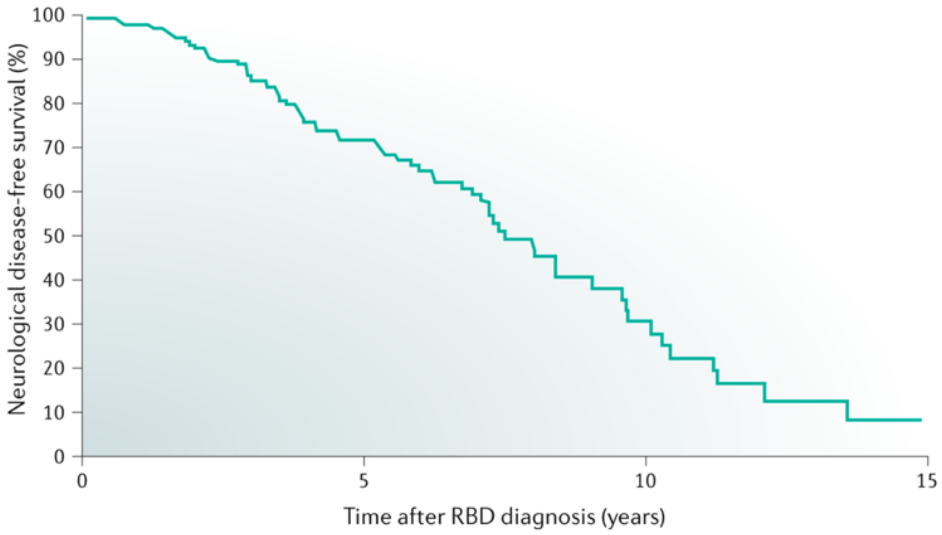


Figure 4 Conversion to synucleopathy following diagnosis of RBD

(Dauvilliers, 2018; Iranzo et al., 2014)

RBD is a parasomnia (abnormal or unusual behavior of the nervous system during sleep) characterised by the loss of muscle atonia during REM sleep: this can cause dream enactment, which can often cause injury both to the individual, and partner. RBD can be idiopathic, or secondary (associated with a synucleopathy such as DLB, PD or MSA). Secondary RBD is now recognised as a manifestation of these neurodegenerative disorders and RBD is the strongest prodromal marker for synucleopathies. When first studied, 40% of RBD patients went on to be diagnosed with PD (or synucleopathies). This number rose to 81% following 13 years post diagnosis. The sleep circuits involved in generating REM sleep muscle atonia/paralysis are yet to be fully mapped, however, the brain nuclei playing an important role in this phenomenon, have begun to be elucidated (Dauvilliers, 2018). This finding that there is prodromal phase that is highly predictable, and detectable, opens a door to understanding the dysfunction that is occurring prior to overt neuronal loss that generates the debilitating motor features of many of these synucleopathies.

However, a caveat meriting mention is the finding that not all patients with PD and DLB present, clinically, with RBD, but will subsequently go on to develop RBD (Dauvilliers, 2018). This difference, some PD/DLB individuals will be RBD first, or RBD after diagnosis, may be attributed to different forms of the diseases. Recent work suggests that PD may develop in distinct pathological axes referred to as a *body first versus brain first PD* — and that these distinct subsets of patients can be distinguished based on their RBD phenotype (Borghammer & Van Den Berge, 2019; Horsager et al., 2019; Postuma & Berg, 2016)

10.09 Treatment

Currently there exists no disease modifying treatment for PD. However, at early stages of the disease, motor symptoms can be significantly reduced by dopamine replacement therapy, in the form of levodopa, a dopamine precursor, coupled with other drugs able to modulate both the activity and biochemistry of dopamine (Figure 5).

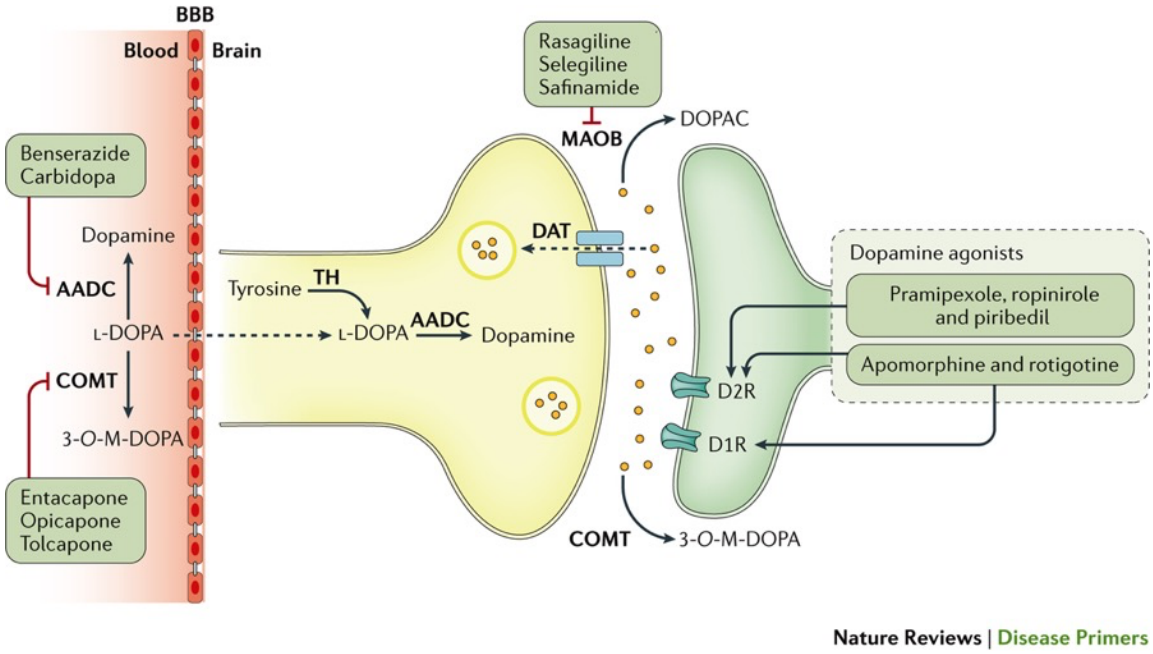


Figure 5 Dopaminergic drug targets in PD

Presynaptic targets include L-DOPA substitution combined with peripherally active inhibitors of aromatic amino acid decarboxylase (AADC) or catechol-Omethyltransferase (COMT). Monoamine oxidase type B (MAOB) inhibitors enhance the synaptic availability of dopamine. Dashed arrow from blood to brain designates blood–brain barrier (BBB) transport of L-DOPA. 3-O-M-DOPA, 3-O-methyl-DOPA; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DOPAC, 3,4-dioxy-phenylacetic acid; TH, tyrosine hydroxylase. (Poewe et al., 2017b)

Levodopa can be combined with carbidopa (which inhibits peripheral metabolism of levodopa), COMT inhibitors as well as MAO-B inhibitors, which all act to increase the levels of dopamine within the brain. Dopamine D2 receptor agonists are also used which are helpful for treating the off periods as well as balancing the slower effects of levodopa. The discovery of dopamine autoreceptors (Carlsson et al., 1972; Kikuchi et al., 2021) able to modify activity on the pre-synaptic neurons, was fundamental to the development of broadly applied D2 agonists. In PD, dopamine agonists have proven antiparkinsonian activity (Brooks, 2000). However, their use has had unintended consequences (that have led to substantial

law suits) leading to pathological gambling, hypersexuality, and compulsive behaviours (Moore et al., 2014). Deep brain stimulation (DBS) can be used when motor symptoms progress or with the loss of clinical efficacy of dopamine replacement therapy (Schwalb & Hamani, 2008). DBS places (an) electrode(s), often in the subthalamic nucleus, providing effective regulation of motor symptoms. Despite being very effective for controlling motor symptoms, DBS does not stop progression of PD, having an interesting and educative darker history (Oliveria, 2018). Given the important role of the dopamine-system in complex behaviour, modulating it does have behavioural consequences that can be deleterious. An important aspect with dopamine therapy is monitoring impulse control disorders. PD does have significant non-motor features that can be addressed pharmacologically, including daytime sleepiness, RBD, nocturnal akinesia, orthostatic hypotension, depression, psychotic symptoms such as hallucinations and delusions, PD-associated dementia and also, drooling (Armstrong & Okun, 2020a; Connolly & Lang, 2014; NICE, 2019; NINDS, 2019).

10.10 Circuit dysfunction — the basal ganglia and beyond

The cardinal motor symptoms in PD are largely attributed to the degeneration of dopamine neurons of the substantia nigra pars compacta (SNc) — a population of neurons acting as critical regulators of basal ganglia circuitry. The basal ganglia (Figure 6) are a group of subcortical nuclei with a primary role in motor control: the striatum and globus pallidus, subthalmic nucleus (STN), SNc, and pedunculopontine nucleus (PPN) (not shown in figure) (Lanciego et al., 2012).

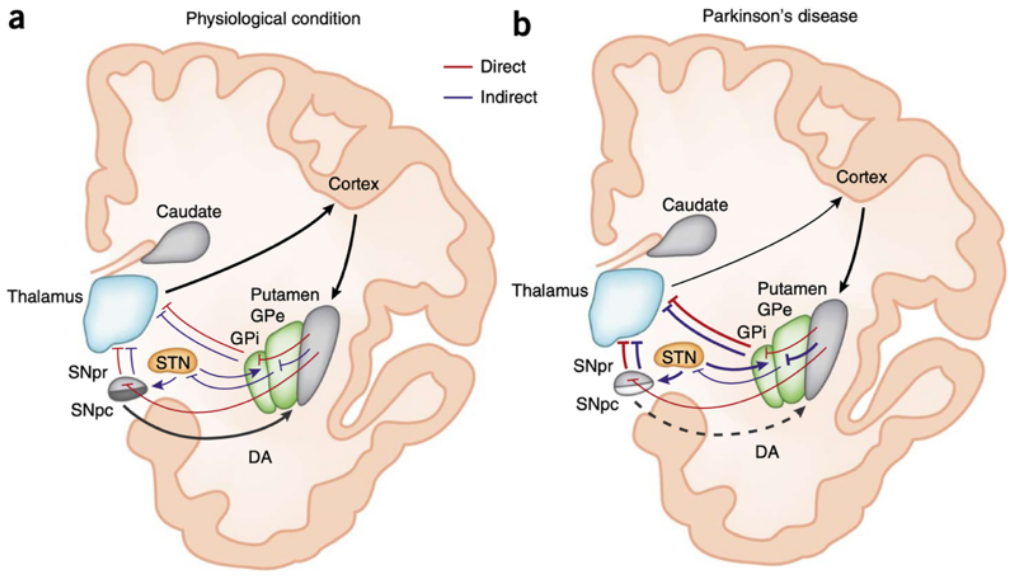


Figure 6 Schematic representation of the direct/indirect basal ganglia pathways (Calabresi et al., 2014)

The model of this network attempts to incorporate multiple circuits associating motor, associative, and limbic territories, in the regulation of behavioural output (in the form of movement and emotion). Figure 6 depicts a current, yet simple, model of the basal ganglia a) in physiological condition, and b) in the context of PD. This model of the basal ganglia posits two main pathways (the direct and indirect pathway) that act in counterbalance to regulate motor output. In the physiological condition, dopamine inputs from the SNc activates D1-expressing striatal medium spiny neurons in the direct pathway and inhibits D2-expressing striatal neurons of the indirect pathway. The output nuclei (the globus pallidus internus and Substantia nigra pars reticulata) project to the thalamus, which then complete the cortico-basal gangliathalamocortical loop. In PD, degeneration of SNc DA neurons reduces DA-mediated stimulation of striatal neurons. The imbalance between the direct and indirect pathways then results in abnormal activation of output nuclei and over inhibition of thalamic neurons projecting to the cortex (Calabresi et al., 2014). Notable work looking at lesions of these nuclei in 1925 (putamen, globus pallidus, and STN)) contributed to both the conceptualization of the model, as well as the association of dysfunction within these circuits and parkinsonian symptoms (Wilson, 1925).

The symptoms in PD cannot solely be explained by the dysfunction of basal ganglia circuitry. As alluded to, multiple other neuronal populations are purported to degenerate. These predominantly deep brain regions play an important role in modulating higher brain regions. The LC, dorsal raphe nucleus and PPN, for example, play an important role in the regulation of sleep: particularly in generating REM sleep, and REM-sleep atonia — thus the prodromal RBD previously described (Dauvilliers, 2018). Other autonomic projecting neurons such as the DMV play an integral role innervating the enteric nervous system, which may — in the context of degeneration — be responsible for prodromal gastrointestinal symptoms (Travagli et al., 2020). The circuitry and role of these systems is further discussed in section 10.20 (Long range projecting neuromodulator neurons).

10.11 Lewy pathology and Braak staging

Unlike other neurodegenerative diseases, PD is not characterized by large macroscopic atrophy of the brain, but rather highly selective restricted neuronal degeneration in select regions. Classical neuropathological findings in PD can be seen in Figure 7. In the PD brain we see loss of neuromelanin pigmentation in the mesencephalon, due to the loss of dopaminergic neurons — in contrast to a healthy individual where we see significant neuromelanin (a biproduct of dopamine oxidation) (Figure 7 A, right

panel). In Figure 7 panels E through G, we see the presence of Lewy Pathology (LP) — both the classical inclusion bodies within the cell body, and neurofibrillary tangles in the neurites (Poewe et al., 2017a).

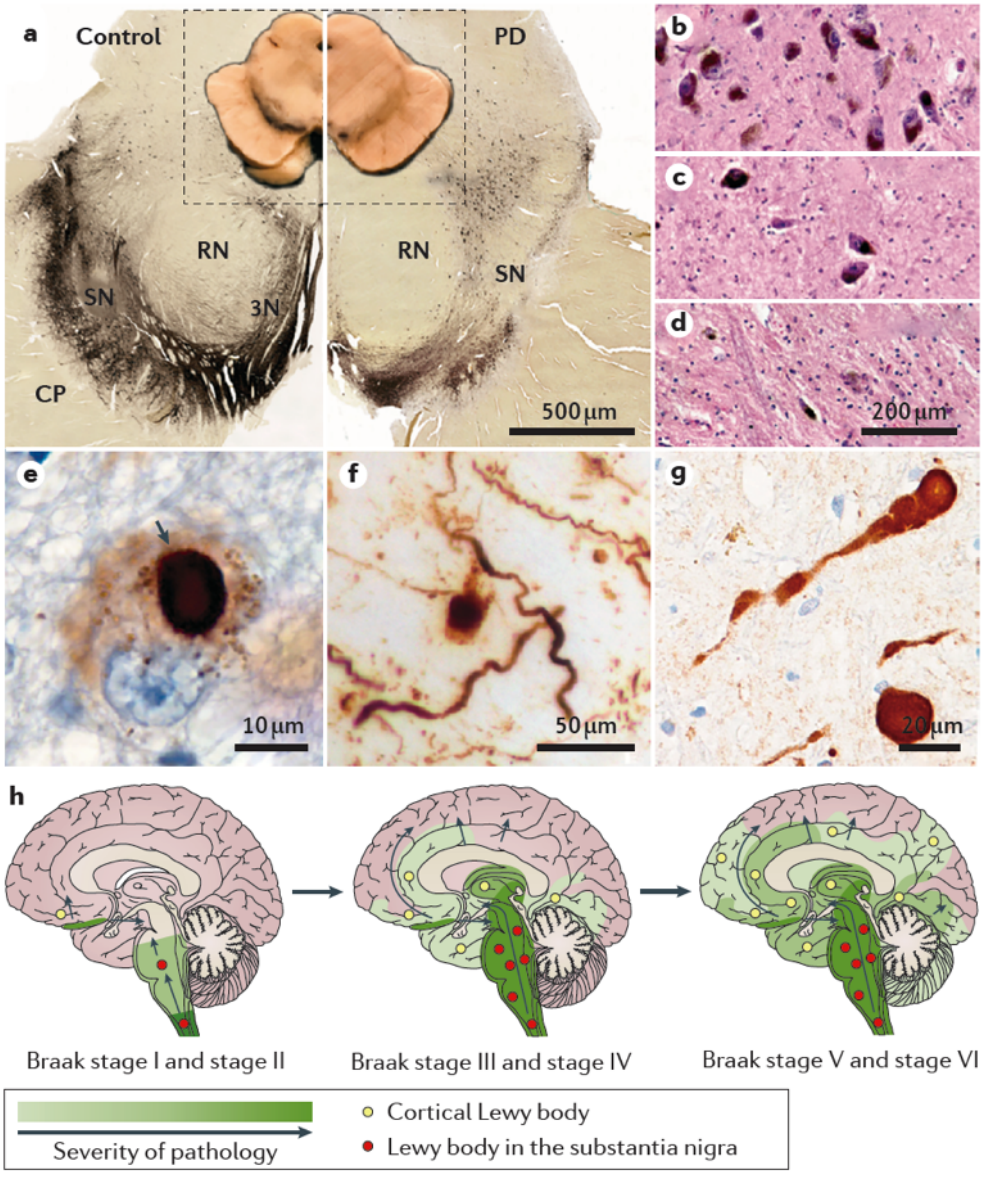


Figure 7 Neuropathology in PD

Parkinson disease (PD) is defined by depigmentation of the substantia nigra (SN) (right panel) compared with control (left panel). Macroscopical (inset) and transverse sections of the midbrain upon immunohistochemical staining for tyrosine hydroxylase, the rate limiting enzyme for the synthesis of dopamine, are shown. Selective loss of the ventrolateral parts of the SNc with sparing of the more medial and dorsal regions is evident in the histological section. b–d | Haematoxylin and eosin staining of the ventrolateral region of the SNc showing a normal distribution of pigmented neurons in a healthy control (part b) and diagnostically significant moderate (part c) or severe (part d) pigmented cell loss in PD. e–g | Immunohistochemical staining of α-synuclein shows the round, intracytoplasmic Lewy bodies (arrow in part e), more

diffuse, granular deposits of α-synuclein (part e and part f), deposits in neuronal cell processes (part f), extracellular dot-like α-synuclein structures (part f) and α-synuclein spheroids in axons (part g). h | The theorized progression of α-synuclein aggregation in PD without Alzheimer pathology. α-Synuclein inclusions occur in cholinergic and monoaminergic lower brainstem neurons in asymptomatic cases (Braak stage I and stage II), infiltrate similar neurons in the midbrain and basal forebrain in those with the motor symptoms of PD (Braak stage III and stage IV), and then are found later in limbic and neocortical brain regions with disease progression (Braak stage V and stage VI)236. 3N, 3rd nerve fibres; CP,cerebral peduncle; RN, red nucleus. (Poewe et al., 2017b)

In 2003, seminal — era-defining work — was published by Eva Braak, Heiko Braak, and Kelly Del Tredici (and colleagues). Continuing from similar work in the context of Alzheimer's disease, they developed a staging system for idiopathic PD (Braak et al., 2003): depicted in Figure 7 panel H. This work has led to several key hypotheses and lines of inquiry in PD. Firstly, this work brought to the fore the selective nature of PD pathology, the progressive nature of this pathology, and the possibility of a transmissibility of this pathology along functionally connected neuronal networks.

In this proposed 6-stage model, they suggest pathology beginning in the brain stem, progressing up towards higher cortical areas as the disease progresses. Firstly, during Braak stages 1 and 2, LP is found in the base of the brain stem, in the dorsal motor nucleus of the vagus (DMV), and in the olfactory bulb. As the disease progresses, through Braak stages 3 and 4, pathology appears in the midbrain area in regions such as the SNc, locus coeruleus (LC), raphe nuclei, and pedunculopontine nucleus (PPN). Finally, in stages 5 and 6 diffuse pathology is found throughout the cortex.

Since first publication, LP has been reported not only in the central nervous system (CNS), but also the enteric nervous system (ENS), and peripheral nervous system (PNS), suggesting that these regions are somehow involved in the degenerative process in PD. LP has been repeatedly confirmed in the DMV, and gut, prior to PD diagnosis — opening up inquiry into prognostic biomarkers, as well as raising attention to a clear prodromal period (Braak & Del Tredici, 2017).

10.12 α-synuclein

> "It therefore is worthwhile to examine the Lewy bodies, find out what they are composed of, and what molecular events precede and accompany their formation. Once we know that, will we be able to prevent Lewy bodies from forming? And if Lewy bodies do not form, will we then have no substantia nigra degeneration and no Parkinson's disease? Perhaps that is too much to expect from Lewy's peculiar cellular inclusions." — Forno, 1987

α-synuclein had been originally identified as a constituent of amyloid found in Alzheimer's disease: the precursor protein for the non–β amyloid component pf AD pathology (Uéda et al., 1993). In 1997, the α-synuclein gene (SNCA) was identified as harbouring a mutation causing a familial form of PD (in an Italian family with penetrance of around 85%). Members of this family had typical PD symptoms, showed LP on autopsy, and had a relatively early onset of disease (46 ± 13 years) (Polymeropoulos et al., 1997). Subsequently, a mere handful of months later, α-synuclein was identified as the main constituent of Lewy bodies in idiopathic PD, as well as in Dementia with Lewy Bodies (DLB) (Spillantini et al., 1997).

The physiological role played by α-synuclein has been elusive. It is largely believed to play an important role within the pre-synaptic domain, having been associated with synaptic vesicle dynamics, mitochondrial function, as well as playing a role in intracellular trafficking of cargo (Poewe et al., 2017b). Synuclein knock out mice, albeit some quantifiable defects, are viable — and have been somewhat unconclusive in revealing the role of the synucleins (Greten-Harrison et al., 2010). More recent work suggests that α-synuclein's role may be in the nuanced regulation of vesicle exocytosis during neurotransmitter release (Sulzer & Edwards, 2019).

The majority of interest in α-synuclein has been exploring the potential pathological consequences of α-synuclein as it "gains" neurotoxic properties during fibrilization: from soluble monomer, to oligomer, and eventually insoluble fibrils. The purported toxic effects of α-synuclein in these non-physiological conformations are manyfold: depicted in Figure 8, where we see the interplay of the major factors thought to contribute to, ultimately, the death of neurons in PD (mediated via interactions with toxic confirmations of α-synuclein). How and why α-synuclein homeostasis is lost remains a key unanswered question. However, it is thought that the physiological control of α-synuclein proteostasis and degradation are carried out by the ubiquitin–proteasome system and the lysosomal autophagy system. Both systems thought to be affected in PD, as well as being systems whose functions decrease as a function of age (Poewe et al., 2017b).

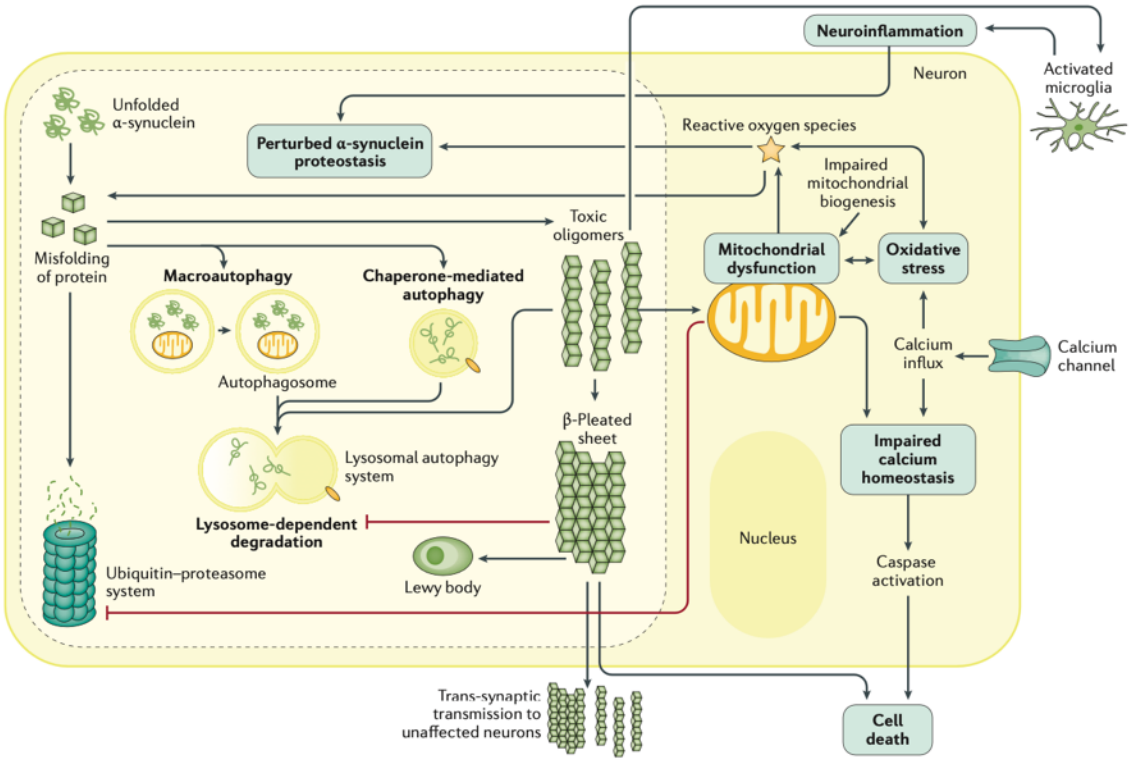


Figure 8 Molecular mechanisms involved in Parkinson disease

Schematic diagram depicting the interaction between the major biological entities in PD, and their contribution to pathogenesis (Poewe et al., 2017b)

Mitochondrial dysfunction has also been suspected as playing an important role in the pathogenesis of PD (Schapira, 2007). The current zeitgeist seems to suggest of a vicious feedback loop whereby the toxic effects of pathological α-synuclein exacerbates mitochondrial dysfunction, that in turn exacerbates pathological α-synuclein (Figure 8).

10.13 Genetics — SNCA and beyond

In the preceding decades, great strides have been made in the genetic origins of PD: uncovering the role played in risk for PD, onset, progression, as well as response to treatment. However, given that we are only beginning to scrape the surface of what is hidden in our genomes, only a fraction of PD heritability has been elucidated.

Most PD cases are sporadic (only 10% of patients reporting a positive family history). Several monogenetic forms of PD have thus far been identified: caused by single mutations which can be inherited

either dominantly or recessively. Though they are rare they are often highly penetrant. Monogenetic mutations account for roughly 30% of familial cases, and 3 to 5% of sporadic cases. (C. Klein & Westenberger, 2012).

Since the first discovery of mutations in SNCA, many other genes have been found to cause autosomal dominant and recessive familial PD. These include the now canonical PD genes LRRK2, PRKN, PINK1, GBA, and DJ-1. A timeline of these discoveries is show in Figure 9, where we see the major discoveries prior to, and following, the era of genome-wide association studies (GWAS). GWAS allow the study of genetic associations to disease phenotypes that are not caused by simple monogenetic factors, but rather loci of risk. These methods allow for the exploration of the contribution of many low-risk genetic variants that, when combined, can cause disease (*Genome-Wide Association Studies (GWAS)*, n.d.). The first GWAS in PD — finding significant genome wide associations — was in 2009 (Simón-Sánchez et al., 2009).

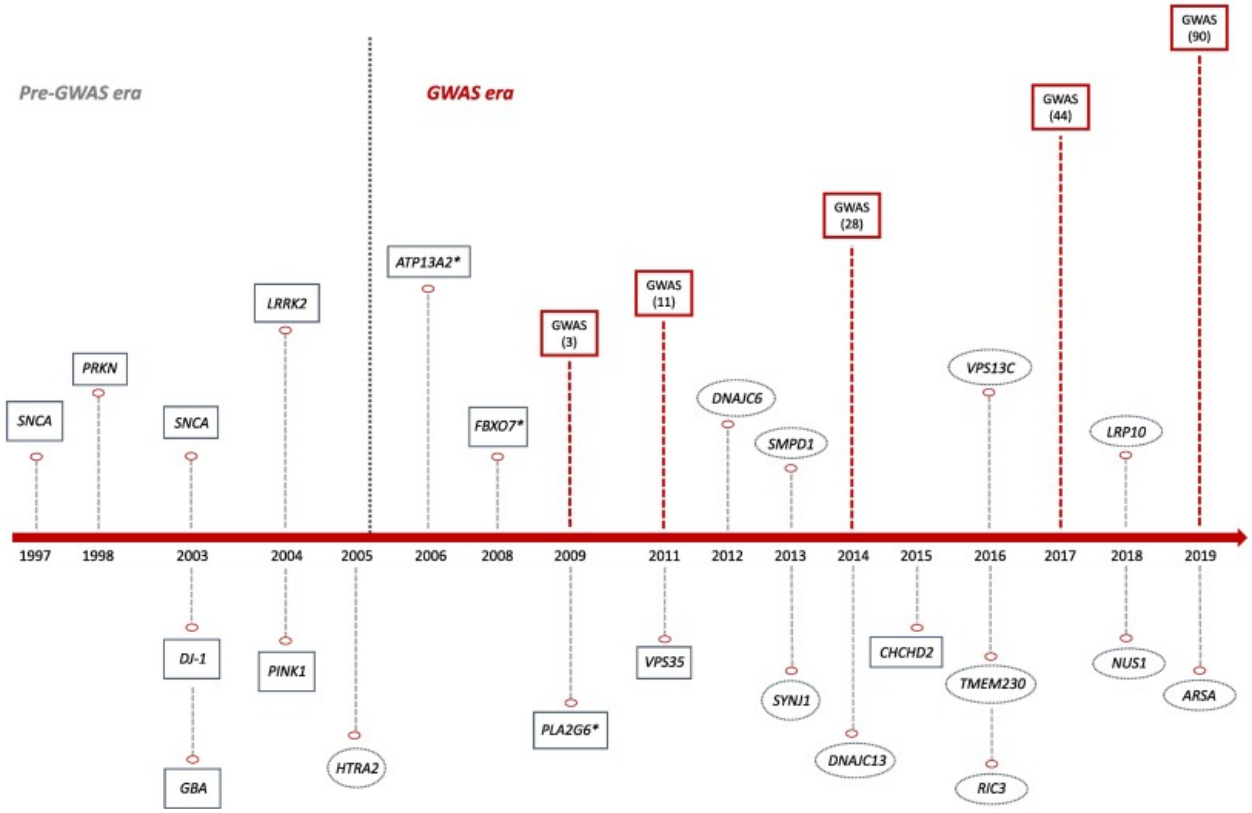


Figure 9 Genetic discoveries in PD

Red squares represent genome-wide association studies and number of discovered risk loci in brackets. * indicate genes associated with atypical parkinsonism related syndromes. Grey circles represent controversial or not widely validated genes linked to typical Parkinson disease (Bandres-Ciga et al., 2020)

The landscape of PD genetics is complex (Figure 10). Mutations in Pink-1, PRKN, SNCA, LRKK2, and DJ-1 have been studied, and have contributed significantly to elucidating the pathophysiological mechanisms suspected to cause disease. Other genes such as GBA (and some variants of LRKK2), which are somewhat more frequent within the population, confer medium risk yet are not disease-causing, per se. The types of mutations found in genes — associated with PD — also varies, with different disease-causing mechanisms existing. For example, when considering SNCA, there is a broad range of common variants, within the healthy population. These variants have a high frequency yet confer relatively lower risk (though not risk-free). We also have missense causative mutations which are relatively rare but elevate risk significantly (depending on their location). And then we can have variants where there is locus duplication and triplications which are associated with high disease linked expression, as well as very high risk for PD diagnosis.

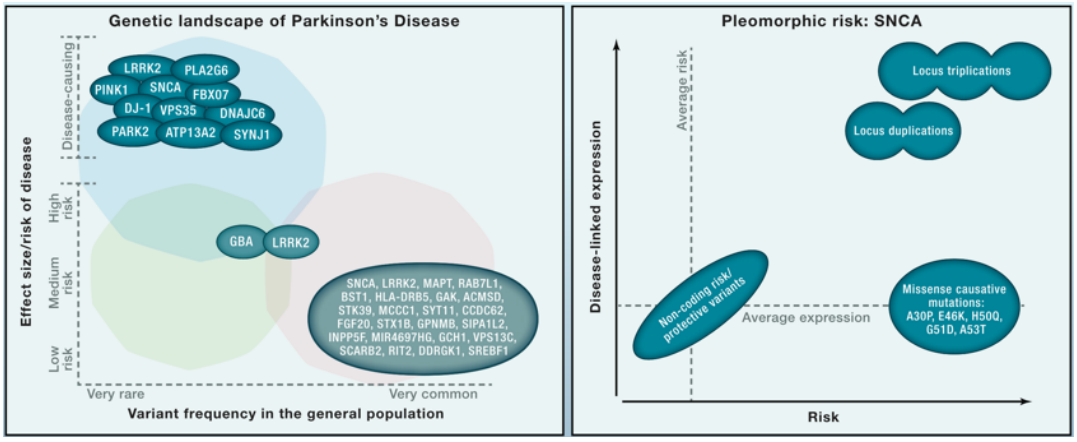


Figure 10 The genetic landscape in PD

(Brás et al., 2015)

The nomenclature for genes is constantly evolving, and for genes associated with PD, there is the PARK nomenclature; spanning from 1-23, describing chromosomal locations that have been demonstrated to have putative links to PD (Kouli et al., 2018). However, this list, and nomenclature, is inconsistent and does not include all known genes associated with PD. Table 3 lists many of the genes, that are somewhat well supported to contribute towards causing PD. Furthermore, the biological processes to which these gene contribute to/are involved in are listed. The biological processes that these genes are involved in has given the filed valuable insights into the core processes of pathology. For example, of the 11 genes listed in Table 3 (*Mendellian genes*), eight are thought to play an important role in mitochondrial function.

Gene (official symbol)	Gene name	Possible pathways/pathological biological processes
Mendellian Genes		
SNCA	Synuclein, alpha	Synaptic function; mitochondrial function; autophagy/lysosomal degradation
PARKIN/PARK2	Parkin RBR E3 ubiquitin protein ligase	Mitochondrial function/mitophagy; ubiquitination; synaptic function
PINK1	PTEN -induced putative kinase 1	Mitochondrial function/mitophagy
PARK7/DJ-1	Parkinson protein 7	Inflammation/immune system; mitochondrial function
LRRK2	Leucine-rich repeat kinase 2	Synaptic function; inflammation/immune system; autophagy/lysosomal degradation
PLA2G6	Phospholipase A2, group VI	Mitochondrial function
FBXO7	F-box protein 7	Ubiquitination; mitochondrial function/mitophagy
VPS35	Vacuolar protein sorting 35 homolog (S. cerevisiae)	Autophagy/lysosomal degradation; endocytosis
ATP13A2	ATPase type 13A2	Mitochondrial function; autophagy/lysosomal degradation
DNAJC6	DnaJ (Hsp40) homolog, subfamily C, member 6	Synaptic function; endocytosis
SYNJ1	Synaptojanin 1	Synaptic function; endocytosis
Risk Genes		
GBA	Glucosidase beta acid	Inflammation/immune system; autophagy/lysosomal degradation; metabolic pathways
Risk Loci		
MAPT	Microtubule-associated protein tau	Microtubule stabilization and axonal transport
RAB7L1	RAB7, member RAS oncogene family-like 1	Autophagy/lysosomal degradation
BST1	Bone marrow stromal cell antigen 1	Immune system
HLA-DRB5	Major histocompatibility complex, class II, DR beta 5	Inflammation/immune system
GAK	Cyclin-G-associated kinase	Autophagy/lysosomal degradation; synaptic function; endocytosis
ACMSD	Aminocarboxymuconate semialdehyde decarboxylase	Tryptophan metabolism; metal ion binding; metabolic pathways
STK39	Serine threonine kinase 39	Inflammation/immune system; protein kinase binding; cellular stress response

SYT11	Synaptotagmin XI	Synaptic function; transporter activity; metal ion binding; substrate for PARK2
FGF20	Fibroblast growth factor 20	Growth factor activity; FGF receptor binding
STX1B	Syntaxin 1B	Synaptic function; SNAP receptor activity; protein domain-specific binding
GPNMB	Glycoprotein (transmembrane) nmb	Integrin binding; heparin binding; cancer pathways
SIPA1L2	Signal-induced proliferation-associated 1 like 2	GTPase activator activity
INPP5F	Inositol polyphosphate-5-phosphatase F	Phosphoric ester hydrolase activity
MIR4697HG	MIR4697 host gene (non-protein coding)	
GCH1	GTP cyclohydrolase 1	GTP binding; calcium ion binding; BH4 metab; metabolic pathways
VPS13C	Vacuolar protein sorting 13 homolog C (S. cerevisiae)	Endocytosis
DDRGK1	DDRGK domain containing 1	Protein binding
MCCC1	Methylcrotonoyl-CoA carboxylase 1 (alpha)	Biotin carboxylase activity; methylcrotonoyl-CoA carboxylase activity; metabolic pathways
SCARB2	Scavenger receptor class B, member 2	Autophagy/lysosomal degradation; receptor activity (lysosomal receptor for GBA targeting); enzyme binding
CCDC62	Coiled-coil domain containing 62	Nuclear receptor coactivator; cancer pathways
RIT2	Ras-like without CAAX 2	Synaptic function; calmodulin binding; GTP binding
SREBF1	Sterol regulatory element binding transcription factor 1	Chromatin binding; cholesterol and steroid metabolic processes

Table 3 Genes and genetic loci independently replicated as being associated with the development of PD

(Brás et al., 2015)

The broadest picture today of genetics in PD identified many novel risk loci: analyzing 7.8M single nucleotide polymorphisms in ~40,000 cases and 1.4M controls, 90 independent genome wide signals in 38 loci were found. Extraordinarily, these analyses addressed ~20–30% of heritable risk in idiopathic PD. And of these, 70 genes were identified being putatively causative. Interestingly, of these genes, their enrichment is predominantly in the central nervous system (CNS) —unlike similar studies in Alzheimer's disease — perhaps suggesting that the pathological mechanisms are in fact highly intrinsic to cells found within the

CNS. GWAS requires very large cohorts, and it is hoped with cohorts reaching 100,000 patients, we will enable us to understand a far greater component of the genetic contributions to PD-risk (Nalls et al., 2019). However, challenges remain as >90% of the identified variants are in noncoding regions, complicating precise identification of risk genes. Furthermore, the homogeneity of subjects in genetic studies has likely hidden useful insights (the majority having been performed in individuals of European ancestry) (Popejoy & Fullerton, 2016). As we expand the diversity of genomes included in these studies, we can only hope that novel insights will emerge, offering to explain a higher proportion of risk. Finally, a key question remains on why these genes — when coding for a risk variant — affect the cell types and tissues that they do. What is it about the biological pathways that are perturbed in the context of a "PD-gene", that affect certain cell types, and not others?

10.14 Pathophysiology

The discoveries related to α-synuclein unravelled important insights into the pathophysiology of PD (Figure 8). However, the subsequent insights made related to the genetic origins of PD have revealed a more comprehensive, yet complicated, understanding of PD pathophysiology. Interestingly, many of these being non-α-synuclein-mediated mechanisms.

In PD, dysfunction of cellular bioenergetics and oxidative stress, and of autophagy/lysosomal processes appear to be clearly implicated in the degenerative process: (Nguyen et al., 2019; Vidyadhara et al., 2019). Within the field, it has become clear that mitochondrial oxidant stress is a key driver of pathogenesis in PD (Jenner, 2003). This idea is supported by three key pieces of evidence: One, genetic mutations that increase mitochondrial oxidant stress, or impair mitochondrial quality control, and lead to early-onset PD (Schapira, 2007). Two, toxins, such as pesticides, that impair mitochondrial function increase the risk of PD (Langston, 2017). And three, mitochondrial function has been shown to decline with age, the largest risk factor in PD (Jenner, 2003).

Many PD-associated genetic variants have also been implicated in lysosome function; and in turn, many lysosomal disease genes are associated with an increases risk for PD (A. D. Klein & Mazzulli, 2018). Furthermore, cellular waste disposal pathways have been found to be enriched in protein interaction network analyses in PD. Some of these genes have also been found to play a role in regulating lysosomal pH (integral to their proper function) and, for example, LRKK2 is significantly implicated in regulating Rabs: proteins playing an integral role in lysosomal function (Wallings et al., 2019). In addition to this, clathrin-mediated synaptic endocytosis, an integral part of the neuronal endolysosomal system, is probably the main early target of PD-causing mutations in auxilin, RME-8, and synaptojanin-1 (Vidyadhara et al.,

2019). As previously mentioned, autophagy plays a crucial role in maintaining proteostasis and a healthy mitochondrial pool: both Pink-1 and Parkin mutations severely perturbing mitophagy. VPS35 and VPS13C mutations both target mechanisms of endolysosomal sorting and trafficking, and mutations in GBA, the most common mutations in PD, compromises lysosomal function. Interestingly, α-synuclein and LRRK2, are classified as members of the endolysosomal system: α-synuclein being enriched, presynaptically, playing a role neurotransmitter release, and subsequent endocytosis of vesicles (Ferguson, 2018; Kulkarni & Maday, 2018; Winckler et al., 2018).

A key question is evident. What is it about all the varied possible pathophysiological mechanisms that cause dysfunction and degeneration of relatively similar cell and neuron sub-populations?

10.15 Immune interactions

In the brain of people suffering from PD, signs of inflammation have consistently been reported. For example, microglial activation was first documented in 1988 (McGeer et al., 1988), implicating immune system activation in PD pathophysiology; including in the SNc (Badanjak et al., 2021). *In vivo*, it has been found that microglia can be activated by α-synuclein aggregates as well as by toxic α-synuclein-containing neurons (Couch et al., 2011), well before cell death. Furthermore, there is data to suggest cellular and humoral immune responses in PD patients (Rockenstein et al., 2018). This includes, altered cytokine profiles in the blood and CSF of PD patients (Boka et al., 1994), as well as the detection of autoantibodies (Brudek et al., 2017). Microglial responses can occur early in the disease — during the prodromal period — with severity correlated with α-synuclein pathology (Stokholm et al., 2017). Genes such as Pink-1 and Parkin are thought to have immune roles, and LRKK2 and GBA are both expressed in immune cells, suggesting that PD pathogenesis involves am important immune component (Tansey & Romero-Ramos, 2019).

Recently, the acquired immune system has been implicated in PD pathogenesis, including T cell infiltration into the SNc. GWAS studies have seen an association of PD with alleles for the major histocompatibility complexes (MHC), and recently, it has been shown that α-synuclein derived peptides can act as epitopes displayed driving helper and cytotoxic T cell responses, perhaps, linking MHC risk alleles, with PD (Sulzer et al., 2017). Pink-1 and Parkin play an important role in the clearance of damaged, and regulation of, mitochondria, playing an important role in mitophagy. In addition, Pink-1 and Parkin have been described to play a role in repressing the presentation of mitochondria derived antigens to the cell surface — implicating a role in the adaptive immune response (Matheoud et al., 2016). These two features uniting two themes in PD pathogenesis — both immune and mitochondrial (dys)function via

known PD-genes causing PD. It has now been shown that, in the presence of mutant Pink-1, following infection by gram negative bacteria, mice go on to develop loss of dopaminergic function in the striatum, with motor dysfunction that is levodopa responsive. Furthermore, these animals have T cells expressing mitochondrial derived antigens (Matheoud et al., 2019).

10.16 Ageing

The most important risk factor for PD (and other neurodegenerative diseases) is age. Today, our understanding of this process suggests that ageing (in the context of tissues and cells) is an irreversible process that is characterized by *nine hallmarks* (depicted in Figure 11): genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, deregulated nutrient sensing, loss of proteostasis, cellular senescence, stem cell exhaustion, and altered intercellular communication. Neurodegeneration may be the tail-end of the spectrum of ageing, with neurons being the cells with an elevated vulnerability to *the hallmarks of ageing*, compounded by an inability to be replaced, unlike other cell/tissue types. One key characteristic of the brain is that it is composed primarily of post-mitotic cells, therefore highly sensitive to *the hallmarks of ageing* — due, in part also, to a relatively higher vulnerability to DNA damage. These hallmarks are biological processes that appear to be implicated in what we currently understand to be pathogenic mechanisms in many neurodegenerative diseases. The question remains however, whether these diseases are accelerated versions of the natural process of ageing (where we can have significantly different biological age for individuals of the same age (Elliott et al., 2021)), or rather, the intersection of risk factors that impact these systems in a selective manner. For example, *loss of proteostasis* may be a biological system/process that becomes unbalanced during ageing — but in the context of an individual who has suboptimal autophagy, ubiquitination, and/or lysosomal function, in combination with variants of α-synuclein with a propensity to fibrilise — becomes pathological earlier in life, irrelevant of the individual's biological age. Mitochondrial dysfunction is also thought to play an integral role in the pathophysiology of PD (and other neurodegenerative diseases), therefore, it is somewhat evident that the normal impairment in mitochondrial function during ageing compounds these existing risks (Hou et al., 2019).

Another key physiological process that increases with ageing is cellular senescence. Cellular senescence is a homeostatic process whereby a cell enters permanent cell cycle arrest in response to a multitude of stimuli (Rodriguez et al., 2015). This can occur both during development and ageing, where an accumulation of senescent cells has been observed in aged human brains (Si et al., 2021). It is associated both with neurogenerative diseases as well as healthy ageing (Martínez-Cué et al., 2020). These stimuli include many of the hallmarks of ageing: telomer shortening, DNA damage, mitochondrial dysfunction, and inflammation (Si et al., 2021). In the context of PD, corelates between DNA damage, ROS production,

α-synuclein deposits, and p16 (tumor suppressor protein that is a cyclin-dependent kinase inhibitor) secretion have been made (to the induction of cellular senescence) (Si et al., 2021). Furthermore, there is strong evidence for cellular senescence playing a key role in PD. For example, PD patients show elevated senescence-associated-β-galactosidase (a key marker of senescence) in their cerebral spinal fluid, and a higer number of senescent astrocytes in the SNc (Martínez-Cué et al., 2020).

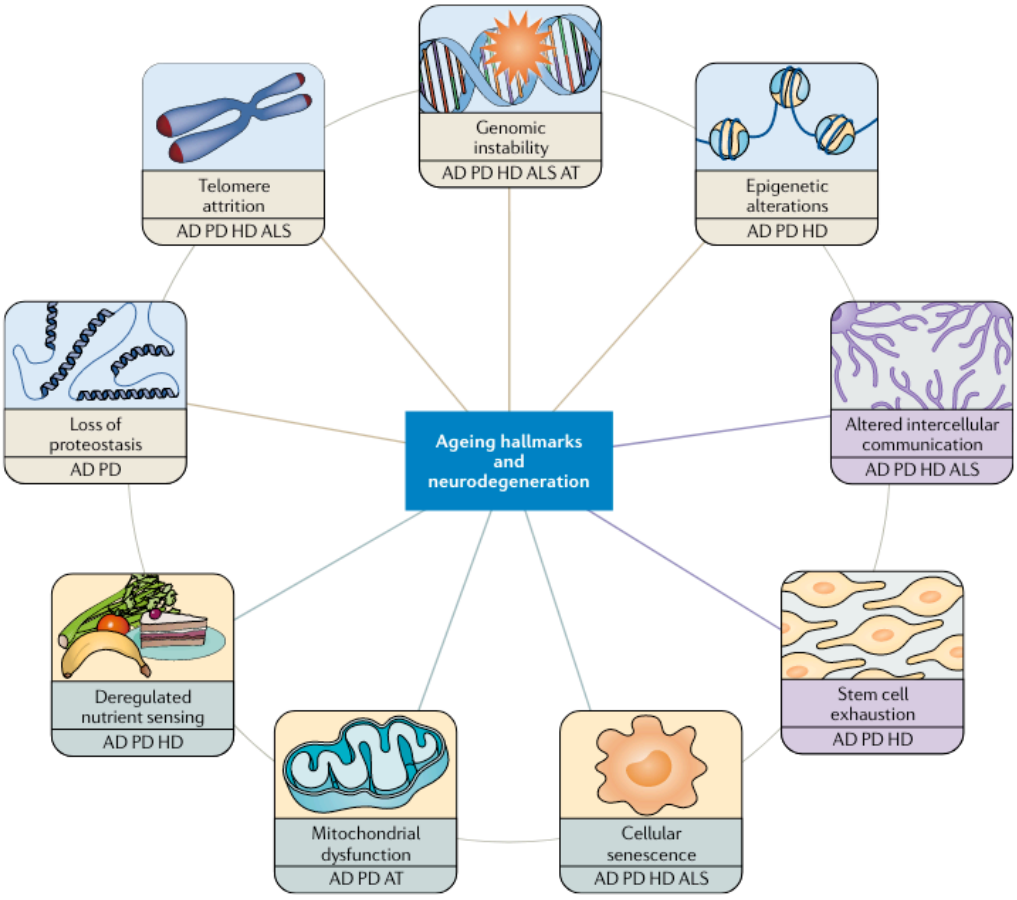


Figure 11 The hallmarks of ageing and correlated neurodegenerative diseases

(Hou et al., 2019)

10.17 Why these neurons? — selective vulnerability in PD

As is common for many neurodegenerative diseases, in PD, pathology and cell loss affect restricted neuronal populations — implicating only a very small proportion of the total number of cells in the brain. Secondly, the progression, and order, seems to follow a relatively stable pattern (Fu et al., 2018).

Multiple observations in the PD literature point towards the identity of neurons that are selectively vulnerable. This includes the initial identification of the SNc as a likely hub of PD degeneration (Lees et al., 2008; Trétiakoff, 1919), to the regions identified by Braak and colleagues (Braak et al., 2003; Braak & Del Tredici, 2017), and further studies on overt cell loss in cases of PD (reviewed in Giguère et al., 2018; Huynh et al., 2021; Surmeier, Obeso, et al., 2017). Furthermore, the identified prodromal period of PD (in particular RBD, constipation, and hyposomnia) (Berg et al., 2021; Heinzel et al., 2019; Postuma & Berg, 2016) all suggest of progressive dysfunction and degeneration in suspected neuronal populations, even prior to overt cell loss.

This raises the following questions: which are the neurons that are degenerating (dysfunction and dying) and in what order does this occur? Why *these* neurons — why does the pathophysiology found in PD cause the selective degeneration of these neurons (that are *PD-vulnerable)*? Are the mechanisms driving cell loss, cell autonomous, or is the disease systemic affecting neurons first? Why and how do the differing risk-factors, genetic mutations, and pathophysiological mechanisms converge on causing the same (roughly) neurons to degenerate?

Finally, many genes identified to be associated with PD tend to be found in gene-sets that appear to be enriched in cells of the CNS (this is not the case, however, for genes such as Pink1, Parkin, LRRK2 and GBA) (Bandres-Ciga et al., 2020; Bryois et al., 2020; Simón-Sánchez et al., 2009). This adds to the evidence for cell-autonomous features being key drivers of neuronal vulnerability in PD. That is to say, there is something about the physiology, function, and/or morphology — that is intrinsic to these cells — that renders them vulnerable to the risk-factors associated with PD.

10.18 “PD vulnerable neurons”

The identity of neurons that are purported to degenerate in PD is controversial and current dogma on this question arises from a diffuse and dispersed literature spanning the best part of the last century (a key theme addressed in detail in Chapters 2 and 3) (Giguère et al., 2018). Currently, it has been suggested that degeneration occurs in many neuroanatomically defined regions (third cranial nerve, amygdala, magnocellular nuclei of the basal forebrain, claustrum, cerebral peduncle, dorsal motor nucleus of the

vagus, intermediate reticular zone, locus coeruleus and subcoeruleus, lateral hypothalamus, the raphe nucleui, paragigantocellular and gigantocellular reticular nucleus, pedunculopontine nucleus, presupplementary motor area, red nucleus (Surmeier, Obeso, et al., 2017)), and occurs progressively, as depicted in Figure 12. The first overt cell loss is found in the SNc, and gradually occurring further up the midbrain area, until numerous nuclei show overt cell loss.

However, evidence for this neuroanatomically defined cell loss is opaque, and the precise neurochemical identity of these neurons is not clear. Currently, the best evidence suggests that "PD-vulnerable" neurons include DA neurons of the SNc (as well as DA neurons of the VTA, to a limited extent — though for the purposes of our current paradigm, and relative to SNc DA neurons, these are "non-PD-vulnerable"), noradrenergic neurons of the locus coeruleus, serotonin neurons of the raphe nuclei, cholinergic neurons of the dorsal motor nucleus of the vagus of the pedunculopontine nucleus, and of the nucleus basalis of Meynert.

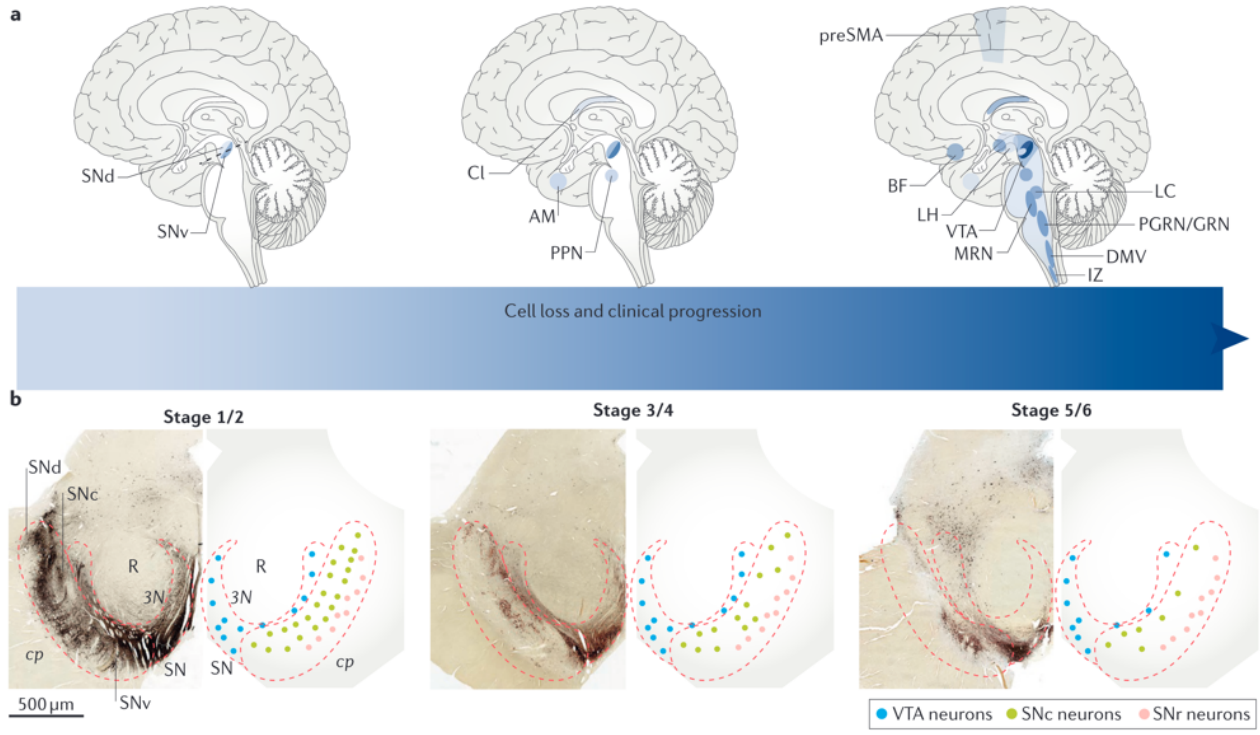


Figure 12 Progressive cell loss as a function of clinical progression in PD

a) The schematics represent the progression of neuronal cell loss following the onset of clinical Parkinson disease (cPD), based on the literature. The anatomical distribution of neuronal loss increases with time, and the darker the colour, the more neuronal loss evident in each region. **b**) Transverse sections of the midbrain, as indicated in the left brain schematic in part a (dotted line), are shown; the normal distribution of tyrosine hydroylase-immunopositive dopaminergic

neurons is shown in the left panels, and the pattern is schematized in the right panels. Heavily pigmented neurons of the substantia nigra pars compacta (SNc) are depicted in green; less pigmented neurons of the ventral tegmental area (VTA) are depicted in blue; neurons of the SN pars reticulata (SNr) are depicted in pink. The initial loss of ventral-tier SNc observed in patients with stage 4 cPD is depicted in the middle panel, with greater cell loss observed over time at later stages, as indicated in the right panel (Surmeier, Obeso, et al., 2017).

10.19 Cell types

Spanish physician and scientist, considered the founder of modern neuroscience/neurobiology, Santiago Ramón y Cajal helped modern science discover neurons as the functional unit of brains — the Neuron Doctrine (Shepherd, 2016); leading to axons, dendrites, synapses, and electrical currents being understood as the fundamental entities that give rise to brain functions (Llinás, 2003; Nemri, 2010).

The categorization of cell types is fundamental to understanding biology, and remains to this day a very much unexplored line of questioning (a majority of research occurs in relatively limited number of cell types (consider the example of HeLa cells: Hyman & Simons, 2011; Svalastog & Martinelli, 2013)). Defining cells by their morphology, physiology, function, and molecular profiles is now in our grasps, and promises to help further understand biology. Major projects include “The Human Cell Atlas” (Rozenblatt-Rosen et al., 2017), an attempt to chart the cell types in the whole human body. And, in 2013, President Obama launched The BRAIN Initiative to “accelerate the development and application of new technologies that will enable researchers to produce dynamic pictures of the brain that show how individual brain cells and complex neural circuits interact at the speed of thought.” (Insel et al., 2013). These modern approaches to define cell types have become highly sophisticated. For example, in a recent description of methods employed : “This was achieved by coordinated large-scale analyses of single-cell transcriptomes, chromatin accessibility, DNA methylomes, spatially resolved single-cell transcriptomes, morphological and electrophysiological properties and cellular resolution input–output mapping, integrated through cross-modal computational analysis” (Callaway et al., 2021).

For the neurons of interest in PD, however, we are still limited to classical cell-type nomenclature — due to the challenges associated with working with tissue/cells/systems of the brain — rooted in the historical nature of their discovery.

10.20 Long range projecting neuromodulator neurons

All neuronal groups suspected to be vulnerable in PD are projecting neuromodulatory neurons. Neuromodulatory neurons (that release neuromodulator neurotransmitters) are a unique category of neurons

involved in regulating brain circuits that control all key brain functions including movement, cognition and emotions.

They differ from most “classical” neurons studied, and taught in textbooks, in their morphology, physiology, and function. They are usually groups within small clusters (nuclei) and project diffusely throughout the CNS, modulating target neurons through synaptic interactions using mainly GABA and glutamate as chemical messengers. Neuromodulator neurotransmitters are typically neuropeptides such as hypocretin, melanin-concentrating hormone, neuropeptide S, and cortistatin, or amino acids derivatives — the monoamines — dopamine, histamine, norepinephrine, and serotonin. In addition, acetylcholine acts as a neuromodulator neurotransmitter within the CNS (vs the PNS) (Bucher & Marder, 2013). Though not addresses in this thesis, neuromodulatory neurons do not exclusively signal with these neurotransmitters, but are often also capable of using the classical fast-acting neurotransmitters (Trudeau et al., 2014). The mode of communication of modulatory neurons with target neurons is best summarized as the following: “any communication between neurons, caused by release of a chemical, that is either not fast, or not point-to-point, or not simply excitation or inhibition.” (Katz, 1999).

Every aspect of neural processing (local circuitry) can be modulated by such neuromodulatory neurons, and all neurons express a range of receptors for these neurotransmitters (Bucher & Marder, 2013; Nadim & Bucher, 2014). Figure 13 outlines the connectivity of major neuromodulatory neurons (to their respective target territories), where these systems give rise to complex behaviours such as decision-making, goal-directed behaviour, emotion, attention, as well as the regulation of sleep/wakefulness states (Avery & Krichmar, 2017). Furthermore, highlighted above target regions, we see diseases and functions that are associated with the neuromodulatory-neuron and target-territory.

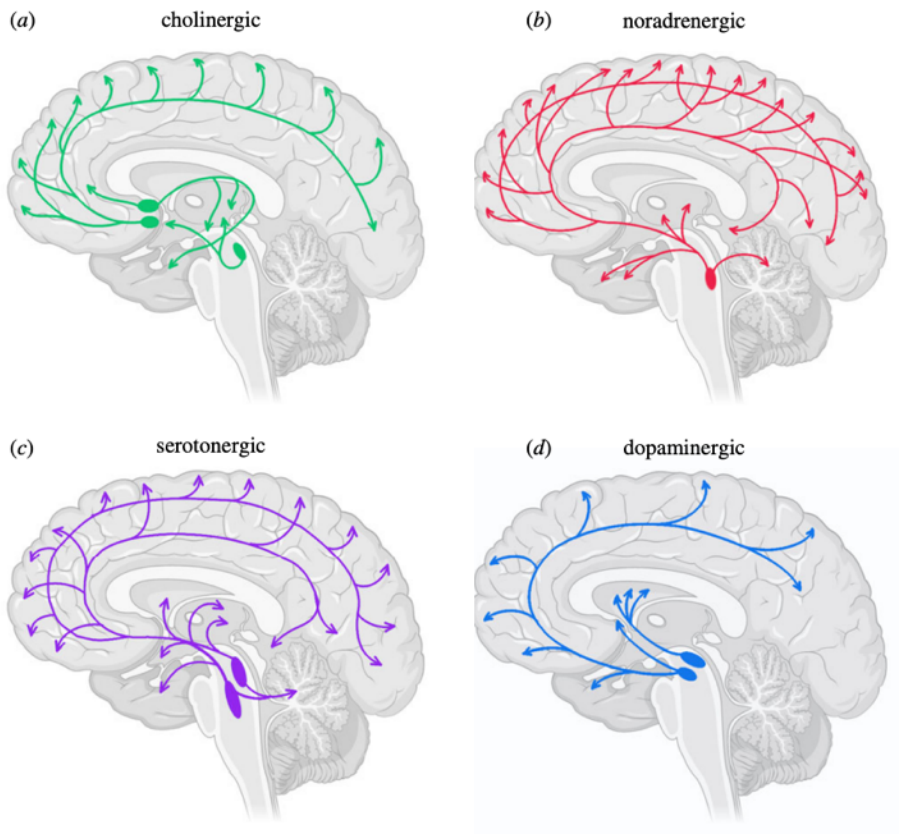


Figure 13 The connectivity of major ascending neuromodulatory neurons

a) Cholinergic neurons of the basal forebrain and pedunculopontine/laterodorsal tegmental complex, **b**) noradrenergic neurons of the locus coeruleus, **c**) serotonin neurons of the raphe nuclei, and **d**) dopamine neurons of the VTA and SNc (O'Callaghan et al., 2021).

How such broadly projecting neurons (single neurons projecting to multiple territories in distant anatomical regions) are able to affect multiple functions, in paradoxical ways, is an open and intriguing question (Chandler et al., 2019). To reach such territories in distal neuroanatomical regions, these neurons project axons across vast distances throughout the brain. Figure 14 depicts the results of tracing experiments of mouse LC neurons as they project across the whole CNS (Chandler et al., 2019).

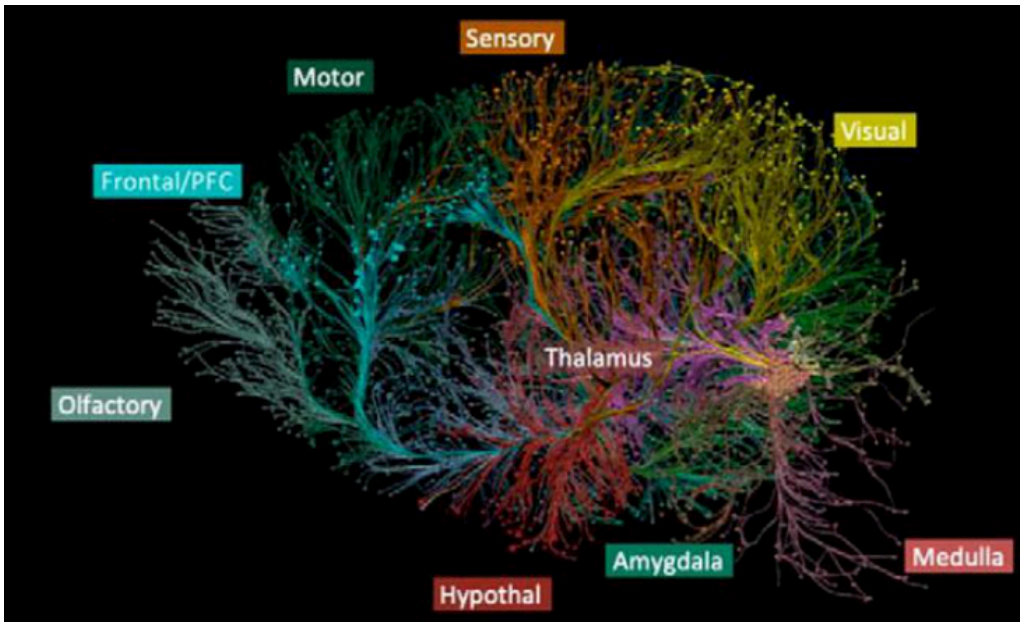


Figure 14 Reconstructions of locus coeruleus neurons projections to the whole CNS, in mouse

(Chandler et al., 2019)

10.21 The axonal domain: arborizations

The vast axonal domain of projecting neurons is best described in DA neurons of the SNc and VTA. These neurons have an incredible capacity to develop a very dense axonal arborization containing numerous neurotransmitter release sites. The first work demonstrating this explicitly date to the late 90s with the work of Parent and colleagues in rats (Gauthier et al., 1999; Prensa & Parent, 2001). Subsequently, the group of Kaneko demonstrated that DA neurons of the SNc, in rat, have an axonal domain totalling around 45cm (Matsuda et al., 2009). Considering the dimensions of non-projecting neurons, usually found in our research being measured in micrometers rather than centimeters (or meters even), begins to depict a picture of the scale of the axonal domain in these specialized cell types.

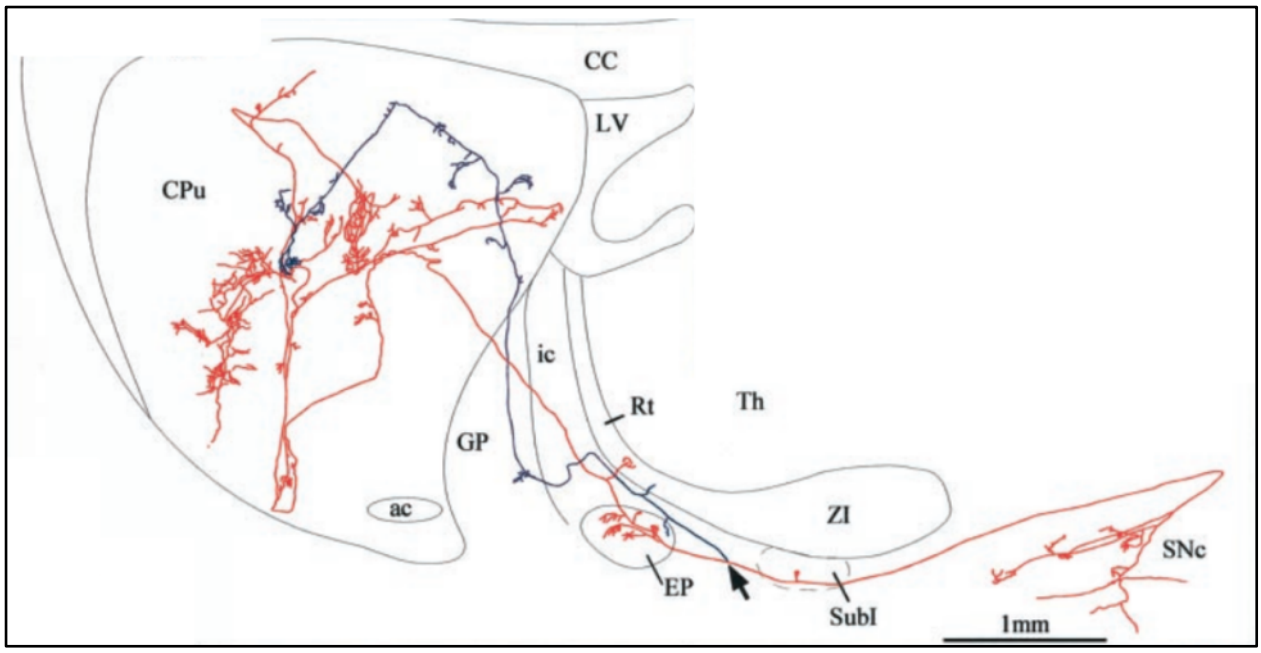


Figure 15 A single nigrostriatal dopaminergic neurons projecting to the striatum in rat.

Arrow indicates main axon bifurcation resulting in two main axons (red and blue) (Prensa & Parent, 2001).

Further work in other projecting neurons has begun to elucidate to the true scale of the axonal domains of these sub-types of neurons. Not only are we beginning to appreciate the total length of these axonal domains, but also the complexity of their branching patterns (Anderson et al., 2002; Braitenberg and Schultz, 1991; Kita and Kita, 2012; Kuramoto et al., 2015; Wu et al., 2014). However, most of this work has involved estimates of average axonal domain volumes, and very few have investigated projecting neuromodulatory neurons with single neurons reconstruction (Economo et al., 2019; Winnubst et al., 2019).

For those that have, data suggests (reviewed in Wu et al., 2014) that in rat, SNc DA neurons have mean axonal lengths of 55–77cm (with 3.4 x 10^9 varicosities per striatum) (Anden et al., 1966; Matsuda et al., 2009). Basal forebrain cholinergic neurons (which include NBM neurons) have estimated axonal arbors totalling between 20–107cm in mouse (Perez et al., 2007; Boncristiano et al., 2002), 60cm in rat (Miettinen et al., 2002; Mechawar et al., 2000; Mengler et al., 2013). In humans, this is estimated to total over 100m (Raghanti et al., 2011; Raghanti et al., 2008a; Rilling and Insel, 1999). Serotonin neurons of the raphe nuclei (the dorsal raphe in particular) are estimated to have total axonal lengths of 60–80cm in rat (Descarries et al., 1982; Cunningham et al., 2005; Vertes and Crane, 1997; Mengler et al., 2013), and anywhere between 170–350m in human (Underwood et al., 2007; Underwood et al., 1999; Rilling and Insel, 1999; Baker et

al., 1991; Raghanti et al., 2008b). No studies have traced noradrenergic LC neurons (however, in the context of this thesis, I have measured single mouse TH+ LC neurons at 7 days *in vitro* totalling over 2–3cm!)

10.22 The axonal domain: neurotransmitter release sites

In the 1960–70s, it became apparent that the electrophysiological responses in local circuits could not be simply explained by excitatory and inhibitory inputs. At the time, nonsynaptic (or asynaptic) neurotransmitter release had only been reported in the PNS, and the "modulation" effect often observed in the CNS was put forth as a potentially important component of synaptic regulation: "nonsynaptic release might provide a basis for diffuse one-cell-to-many communication, but it might also simply be a means of sending the transmitter to a broader area of a single neuron than occurs in typical synapses" (Dismukes, 1979). These ideas put forward were key in reconsidering the notion that direct pre-synaptic domain, to post-synaptic domain communication is uniquely central to neuronal function. Terms initially used to describe the phenomena included *neurohumor*, *neurohormone*, *neuroregulator*, until the emergence and recognition of the important role played by projecting *modulatory/modulator* neurons. Though the possibility of asynaptic release being plausible had existed for some time, it was only until the work of the late Laurent Descarries, in the mid-70s, that this important role was veritably established (described and reviewed: Fuxe, 2000; Katz, 1999; Trudeau, 2012).

Termed "diffuse" or "volume" transmission, this alternative form of neurotransmission — used by neuromodulator neurons — involves non-synaptic axon terminals that release neurotransmitter acting on target cell metabotropic receptors at distances of tens of microns. This is in contrast to the fast-acting classical synapses that are in direct apposition between hyperspecialized pre- and post-synaptic domains (Agnati et al., 1995; Descarries & Mechawar, 2000; Südhof, 2012). This distinction has been thoroughly studied, especially within the context of DA neurons (Figure 16), where we now have robust models of the structure and function (Liu et al., 2021). In addition, it has now become apparent that even for these now classical *asynaptic* varicosities, their synaptic or asynaptic nature remains to be elucidated, with conflicting data reported on the proportion of varicosities exhibiting these properties (Banerjee et al., 2020; Ducrot et al., 2021).

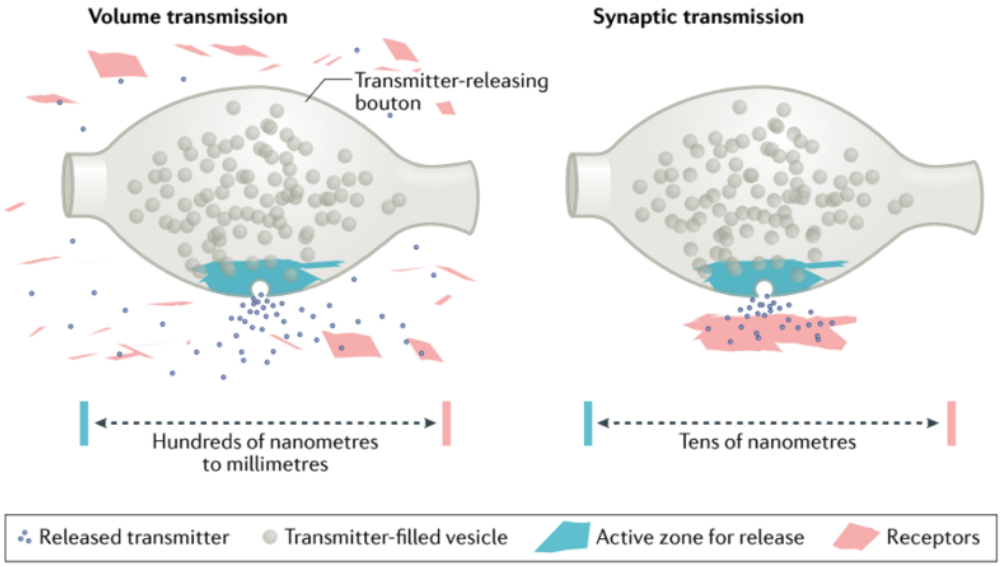


Figure 16 Neurotransmitter release by modulatory neurons by varicosities (transmitter-releasing bouton)

Left. *Volume transmission* relies on the diffusion of transmitters in the extracellular space, and the receptors are only loosely coupled with the release sites. Often, specialized active zone-like release sites mediate neuromodulator secretion. **Right**. *Synaptic transmission* relies on tight spatial coupling between the active zone and receptor clusters, which are often aligned with one another at the subsynaptic scale. (Liu et al., 2021).

However, the molecular differences between synaptic and asynaptic (Figure 16) neurotransmitter release sites are yet to be fully resolved (Banerjee et al., 2020; Ducrot et al., 2021; Liu et al., 2018), though the make-up of the pre-synaptic domain remains relatively consistent (Südhof, 2018, 2021). What is most striking from a cell-type perspective, and therefore morphology and function, is the density of these neurotransmitter release sites along axonal domains of these projecting neuromodulatory neurons.

In humans, though data does not exist, it has been estimated that a single SNc DA neuron may have somewhere between 1–2 million neurotransmitter release sites (Bolam & Pissadaki, 2012). These extremely long, highly branched, and neurotransmitter-release-site dense axons, therefore, must present the cell an immense biological challenge in terms of growth, maintenance, and repair, especially as related to membrane synthesis and axonal transport. Furthermore, the bioenergetic burden must be even more considerable when one considers the unique physiological properties that define neurons.

10.23 Cell-autonomous features of PD-vulnerable neurons

PD-vulnerable neurons can also be defined by their physiological properties. Though comparative work across PD-vulnerable neurons is lacking, a significant amount of physiological phenotyping in DA neurons (often comparing the SNc to VTA DA neurons) has given us clues into the physiological properties of these cells that may render them vulnerable in PD (with some limited validation in other PD-vulnerable neurons such as the LC, DMV and PPN).

These projecting neurons are autonomous pacemakers, with slow rhythmic spiking occurring at between 1–10 Hz (varying across neuron types). These spikes are broad, accompanied by large oscillations in intracellular calcium concentrations, driven by voltage-dependent Cav1 Ca2+ channels, also known as L-type Ca2+ channels (Zampese & Surmeier, 2020). In addition to these oscillations, these neurons appear to have a relatively low capacity for buffering calcium, as shown for SNc DA neurons.

ATP, in neurons, is produced mainly by mitochondrial oxidative phosphorylation (Pacelli et al., 2015). During pacemaking, the elevated intracellular calcium drives mitochondrial uptake of respiratory substrates by acting on receptors on the inner mitochondrial membrane. This stimulates ATP production by complex V. This mechanism helps ensure that ATP is always available for this continuously active firing pattern. Unfortunately, and it is thought to be an integral contributor the vulnerability of these cells, this in turn drives the production of reactive oxygen species (Surmeier, Obeso, et al., 2017; Surmeier, Schumacker, et al., 2017). In addition to this "feed-forward" mechanism, the management of this extra calcium within intra-cellular compartments is carried out by ATP-dependent calcium pumps, adding to the burden of locally synthesized ATP (Canavier et al., 2016). As depicted in Figure 17, these mechanisms, coupled with previously mentioned pathophysiological pathways (Figure 8), all converge in exacerbating one another. The elevated intracellular calcium, associated with the activity of these neurons, has also been suggested to contribute to the fibrilization of α-synuclein. And, given α-synuclein's presence on the pre-synaptic membrane, and the large number of neurotransmitter release sites, it is unsurprising that the projecting neurons are most vulnerable to the intersection of all these unique features.

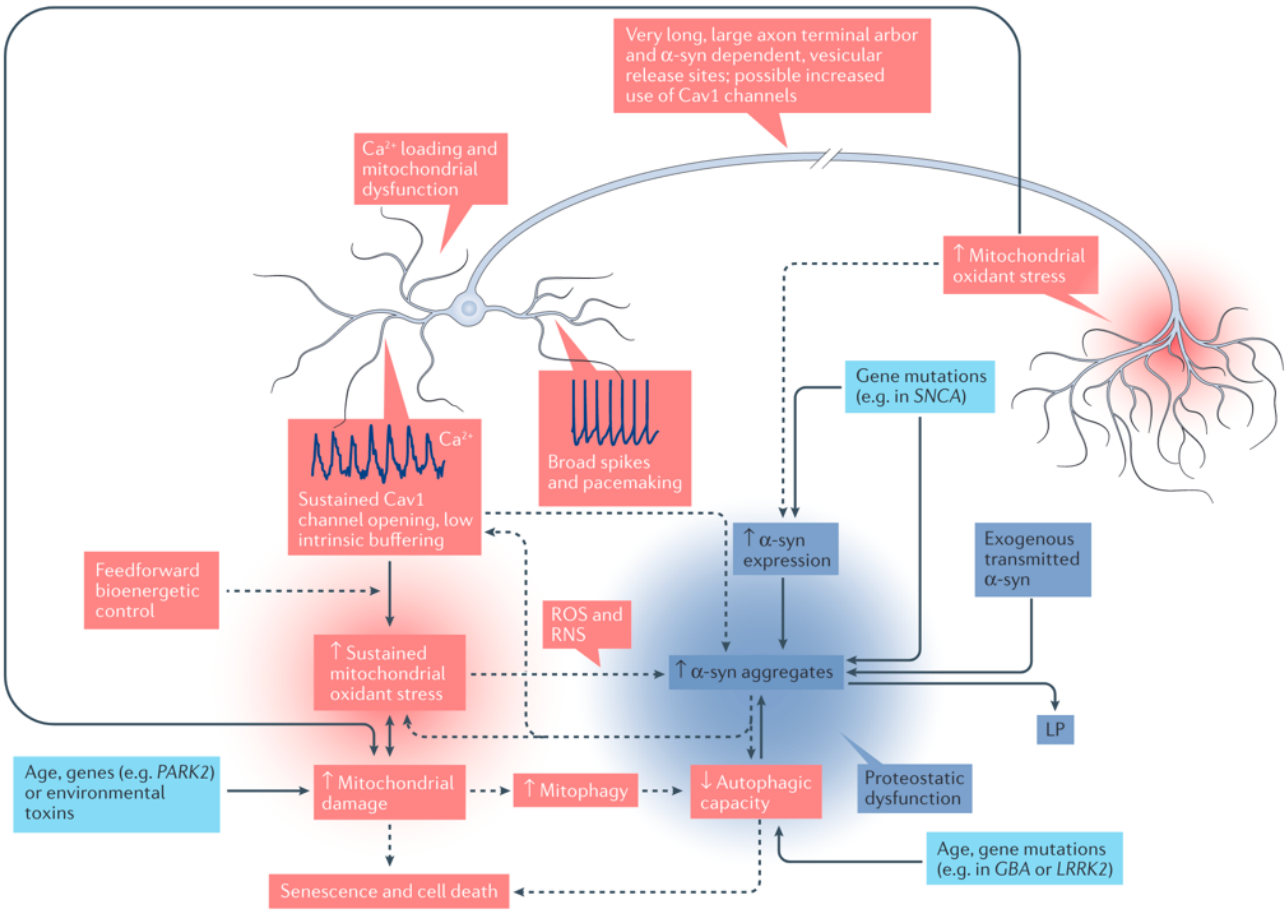


Figure 17 Cell autonomous and non-cell autonomous factors driving degeneration in vulnerable neurons in PD.

Schematic diagram summarizing how cell autonomous factors, non-cell autonomous factors and their interaction can contribute to neurodegeneration and cell death in PD (Surmeier, Obeso, et al., 2017).

In their now classic paper "Living on the Edge With Too Many Mouths to Feed: Why Dopamine Neurons Die", Bolam & Pissadaki (2012) put forward the hypothesis that the selective vulnerability of neurons in PD cannot be understood without a strong and deep consideration for the unique nature of the axonal domain of these projecting neurons. Though many groups are addressing the questions lain out by this seminal paper (summarized in Wong et al., 2019), comparative work across multiple suspected PD-vulnerable neurons is limited. This is essential to refine this hypothesis and begin to truly start testing it. This hypothesis can be stated as following: the purely energetic demand of maintaining a vast axonal domain places a cell close to their maximal capacity, during normal physiological conditions. Therefore, with any extra demands these cells find themselves in an extra-homeostatic condition, causing deleterious consequences. Robust theoretical evidence for this hypothesis involves estimating the energetic

requirements for signal propagation in these highly branched axonal domains. These models do exclude, however, the energetic demands related to the giant axonal domains. Many of the cellular processes involved in cellular transport are ATP-dependent, suggesting that transporting cellular cargo to distal cellular compartments likely contributes to the energetic demands of these neurons (Mandal & Drerup, 2019). In Figure 18 we see this summarised, where (panel B) we can see the projected number of ATP molecules required to restore the membrane potential (given these cell's electrophysiological profile). Importantly, we see that the required ATP demands only begin to rise exponentially at branching levels of 10–13. This being (as per the VTA and SNc labels), a key difference between SNc and VTA neurons. It is suggested, with some evidence, that this significantly differentiates SNc and VTA DA neurons at a morphological level, and may be a key factor in the differential vulnerability of these two DAergic neuronal populations (Giguère et al., 2019).

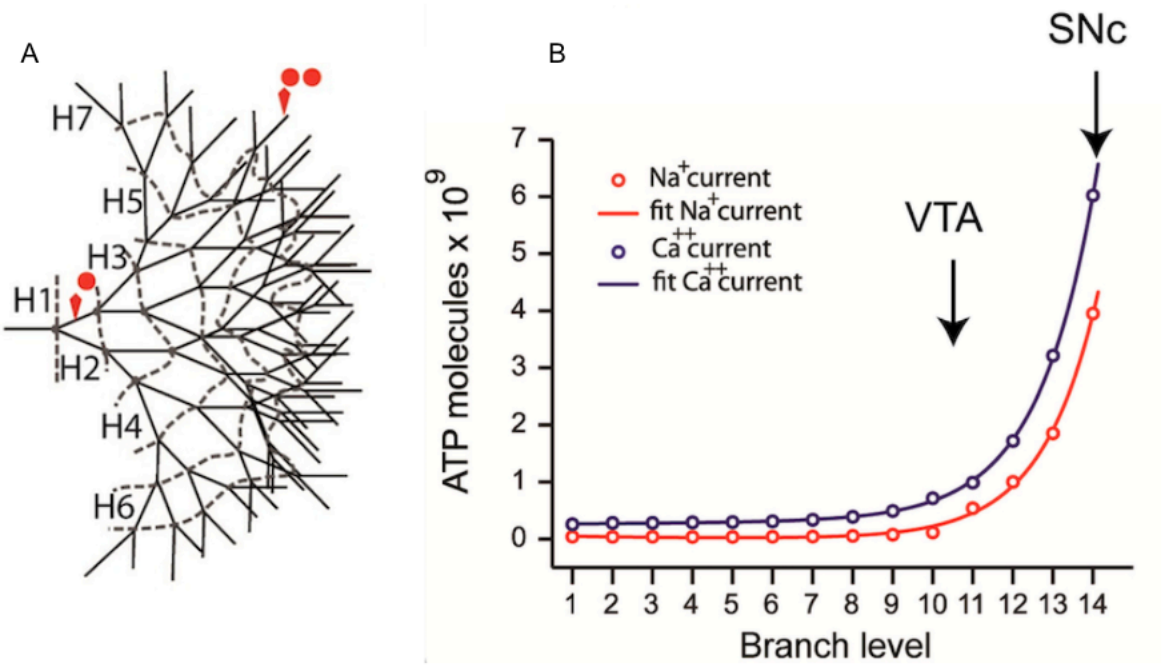


Figure 18 Modelling the exponentially increasing energetic costs of signal propagation in DA neuron axonal arbors

a) representative axonal arbors used in modelling **b**) number of ATP molecules required to restore membrane potential increases exponentially with respect to the length and branch level of the axon both as a result of calcium influx (blue) and sodium influx (red). Arrows indicate the average branching levels for VTA and SNc DA neurons. *Adapted from* (Canavier et al., 2016).

Direct evidence for this hypothesis is scarce. However, work in our group was able to provide the first direct evidence for a direct link between the size and complexity of the axonal domain of DA neurons,

their vulnerability to PD-relevant stress assays, and their ATP production/consumption (summarized Figure 19).

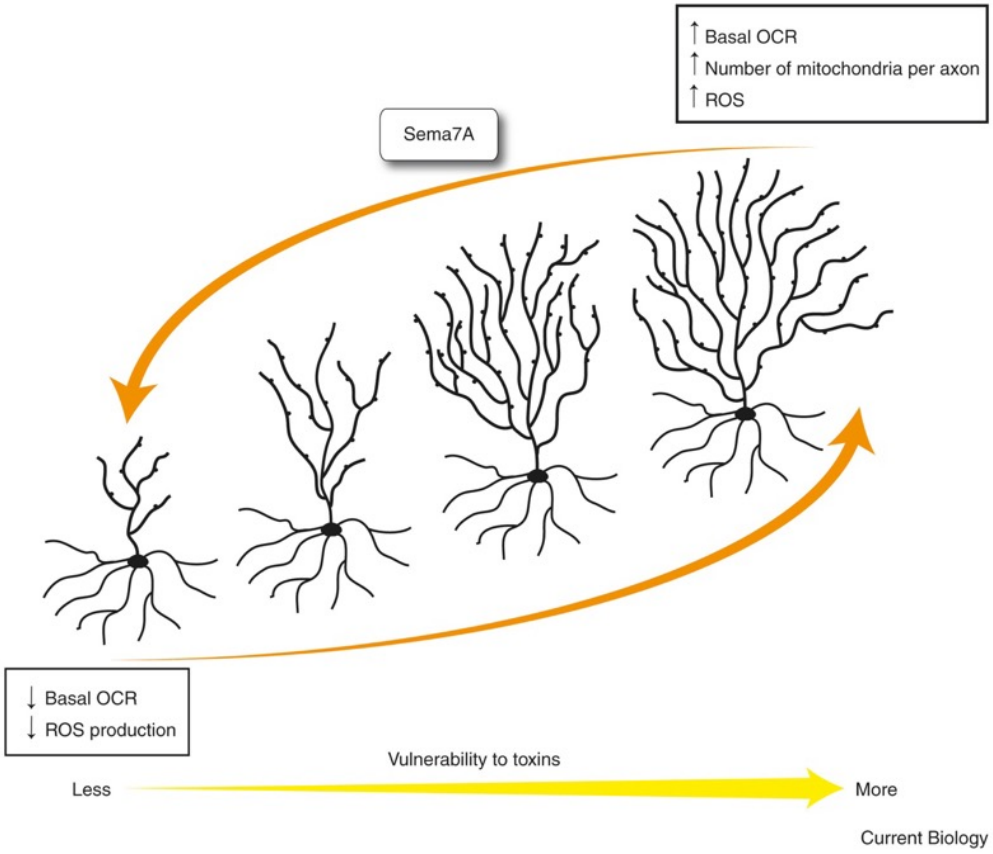


Figure 19 Summary of findings in Pacelli et al., 2015

Increased axonal arborization correlates with basal oxygen consumption rate (OCR), and increased reactive oxygen species (ROS) production. This morphology and physiology correlates with the vulnerability of SNc DA neurons to some mitochondrial toxins MPP+ and rotenone (Franco-Iborra & Perier, 2015; Pacelli et al., 2015).

In this work, Pacelli and colleagues (Pacelli et al., 2015) showed that SNc DA neurons consumed oxygen, at basal conditions, at a higher rate than DA VTA and olfactory (OB) DA neurons, and their capacity to increase ATP production was already saturated (unlike OB and VTA DA neurons) — suggesting that they were already producing a maximal amount of ATP possible. In turn, SNc DA neurons had axonal arborization that were significantly larger, and more complex. Furthermore, SNc DA neurons were most vulnerable to cell stress induced by both MMP+ and rotenone, two mitochondrial toxins associated with environmental causes PD-like clinical symptoms. Treating these cells with the axonal guidance factor Semaphorin 7A caused a reduction in the size and complexity of the axonal arbor of SNc DA neurons, and

resulted in a decrease in basal oxygen consumption, and improved survival in toxin assays. This providing the first causal evidence that there may be a direct link between the vulnerability of these neurons, and the size and complexity of their axonal arborizations.

10.24 Questions in this thesis

The identity, both anatomical and neurochemical of neurons that are degenerating in PD, versus those that are not, underpin our model of selective vulnerability. How robust is this evidence to support the claims in the PD field?

In Chapter 2, we show that the primary evidence for these claims do not support the temporal order, neurochemical identify, and relative loss, of neuronal populations purported to be degenerating in PD. Given the weakness of the evidence has our field begun to address this, following our analyses? In Chapter 3 we describe the claims made citing our systematic review to highlight that many authors do not integrate the message that we have put forward.

Subsequently, in Chapter 4, given prior work in our group showing important differences in vulnerability of SNc and VTA DA neurons, *in vitro*, we aimed to perform similar comparisons across multiple populations of projecting neurons thought to be vulnerable in PD. How does the vulnerability, to PD-relevant assays, of multiple projecting neuromodulatory neurons — both PD-vulnerable and non-vulnerable — compare, and how might this be understood in terms of the morphology and physiology of the axonal domain?

We hypothesized that overt differences in the growth of the axonal domain of would be evident *in vitro*, and that PD-vulnerable nuclei would show larger and more complex axonal domains. Furthermore, we believed that we would observe elevated vulnerability to a cell stress assay in PD-vulnerable neurons, coupled with elevated generation of reactive oxygen species, due to their larger and more complex axonal domains.

11 Chapter 2 — Review on cell loss in the Parkinson's Disease literature

Overview

This review was born out of the observation that, very often, references referring to cell loss often resulted in one going in circles, following citations, trying to find primary data on cell loss. It became apparent that the quality of primary data was relatively weak, and that very few studies in confirmed cases of PD were done in a manner that enabled correlation between relevant clinical progression, and overt documented cell loss. Furthermore, it became apparent the neurochemical identity of neurons counted has been often overlooked, and our assertions on the specific temporal order of neuronal cell loss and degeneration are founded on opaque data.

Contributions

Samuel Burke: conception, data collection, analysis, writing of manuscript.

Nicolas Giguère: data collection, analysis, writing of manuscript.

Louis-Eric Trudeau: conception, providing resources, supervision, writing of manuscript.

Published in Frontiers in Neurology https://doi.org/10.3389/fneur.2018.00455

11.01 On cell loss and selective vulnerability of neuronal populations in Parkinson's disease

Samuel Burke Nanni*, Nicolas Giguère*, and Louis-Eric Trudeau (* co-first authors)

11.02 Abstract

Significant advances have been made uncovering the factors that render neurons vulnerable in Parkinson's disease (PD). However, the critical pathogenic events leading to cell loss remain poorly understood, complicating the development of disease-modifying interventions. Given that the cardinal motor symptoms and pathology of PD involve the loss of dopamine (DA) neurons of the substantia nigra pars compacta (SNc), a majority of the work in the PD field has focused on this specific neuronal population. PD however, is not a disease of DA neurons exclusively: pathology, most notably in the form of Lewy bodies and neurites, has been reported in multiple regions of the central and peripheral nervous system, including for example the locus coeruleus, the dorsal raphe nucleus and the dorsal motor nucleus of the vagus. Cell and/or terminal loss of these additional nuclei is likely to contribute to some of the other symptoms of PD and, most notably to the non-motor features. However, exactly what regions show actual, well-documented, cell loss is presently unclear. In this review we will first examine the strength of the evidence describing the regions of cell loss in idiopathic PD, as well as the order in which this loss occurs. Secondly, we will discuss the neurochemical, morphological and physiological characteristics that render SNc DA neurons vulnerable, and will examine the evidence for these characteristics being shared across PD-affected neuronal populations. Some of the insights raised by focusing on the underpinnings of the selective vulnerability of neurons in PD might be helpful to facilitate the development of new disease-modifying strategies and improve animal models of the disease.

11.03 Introduction

Parkinson's disease (PD) was first described two centuries ago in An essay on the shaking palsy (3). Since then, great strides have been made in understanding the disease basics. However – as with many other neurodegenerative disorders – there is still no disease modifying treatment for PD. Unfortunately, progress has been slow, and a thorough understanding of the pathological processes has been elusive.

PD as a clinical diagnosis is characterized by the detection of significant motor deficits (including bradykinesia, resting tremor and rigidity) due, in large part, to a loss of dopamine (DA)-containing neurons of the substantia nigra pars compacta (SNc). The SNc is a neuronal population projecting to the caudate

and putamen and is critical for regulation of basal ganglia circuitry. At clinical presentation, it has been estimated that 40 to 60% of SNc DA neurons have already degenerated (4, 5). The clinical features of the disease are diverse and include substantial non-motor features including, autonomic and olfactory dysfunction, constipation, sleep disturbances, depression, and anxiety (7-9).

The diagnostic criteria for PD have been recently re-defined by the International Parkinson and Movement Disorder Society (MDS), with the MDS Clinical Diagnostic Criteria for Parkinson's disease (MDS-PD Criteria (6)). A diagnosis is made when there is documented parkinsonism (defined as bradykinesia, with tremor at rest and/or rigidity), followed by the exclusion of other possible causes of parkinsonism, and with additional supporting criteria, including olfactory dysfunction or cardiac sympathetic denervation (See (6)). The recent nature of this re-evaluation illustrates both the heterogeneity of PD expression, and the difficulties encountered in defining it.

In ~70% of the 'clinically typical PD cases', the hallmark pathological finding is the presence of Lewy pathology (LP) in the SNc (7, 8) – however, LP is also found across the central, peripheral, and enteric nervous system (CNS, PNS, and ENS) (9). This includes both Lewy bodies and Lewy neurites: both similar cellular inclusions, formed predominantly of aggregated alpha-synuclein, but also including a large number of different molecules, proteins and organelles, such as ubiquitin, tubulin, neurofilaments, lipids, and mitochondria (10).

In considering the broad localization of LP and the origins of the various symptoms of PD, a critical point to consider is the dysfunction and loss of neurons in regions of the CNS and PNS, other than the SNc. There have been, indeed, many studies concluding that cholinergic neurons in the pedunculopontine nucleus (PPN), noradrenergic neurons of the locus coeruleus (LC), cholinergic neurons of the nucleus basalis of Meynert (NBM) and of the dorsal motor nucleus of the vagus (DMV), and serotonergic neurons of the raphe nuclei (RN) are lost in PD. The strength of the evidence for actual neuronal cell body loss in these regions is highly variable and one of the questions addressed in the present review. The fact that the diagnostic criteria for PD have over time been refined adds another layer of complexity to the task of identifying the origin of the diverse symptoms of PD. Presently, PD is classified into either primary or secondary subtypes. Primary parkinsonism includes genetic and idiopathic forms of the disease and secondary parkinsonism includes forms induced by drugs, infections, toxins, vascular defects, brain trauma or tumors or metabolic dysfunctions. This second subtype of PD is also sometimes called atypical parkinsonism when concomitant to progressive supranuclear palsy, multiple system atrophy or corticobasal degeneration, for example.

Since pathology is likely to emerge through different processes depending of PD subtypes, and since modern classification was non-existent when a substantial part of the research literature was produced,

attempting to reach clear general vision of various pathophysiological markers and their link to disease progression for each sub-type of PD presents a significant challenge. This review will primarily focus on idiopathic PD, since this category represents more than 70% of cases and is likely to represent most of the subjects examined in studies where PD type was not provided.

Another main hurdle in PD research is that the chain of events that leads to the death of neurons is still not clear. The fact that pathology is thought to begin years/decades before the appearance of symptoms might, in part, explain this lack of progress.

PD has been considered to exist as either a strictly monogenetic or environmentally-triggered disease, as well as a mixture of the two. The pathological mechanisms at the core of each form have been proposed to converge in causing cellular stress such as mitochondrial dysfunction, perturbed proteostasis and elevated oxidative stress. A major conundrum is that at first glance, these factors alone fail to explain why PD pathology is restricted to very limited subsets of brain nuclei. Therefore, a key question is what do these PD sensitive neurons have in common and what is it about them that renders them more vulnerable compared to neurons from other brain regions?

A better understanding of the fundamental nature of cell loss and cellular dysfunction in the parkinsonian brain is required to develop critically needed, novel, therapeutic strategies. In this review, we aim to re-evaluate the evidence for cell loss in PD, then to highlight the common characteristics that could explain their selective vulnerability.

11.04 Physiopathology of Parkinson's disease

The focus on SNc DA neurons has brought significant advances in our understanding of PD pathophysiology, as well as of the signaling pathways that lead to DA neuron death. Studies using DA neuron selective toxins such as 6-OHDA and MPTP, as well as investigations of gene products mutated in familial forms of the disease (including alpha-synuclein, Parkin, Pink1, LRRK2, DJ-1 and GBA1), have been instrumental to better understand some of the key dysfunctional processes implicated in the disease. These include protein clearance (11-13), mitochondrial turnover (14-16), ROS management (17, 18) and inflammation (17, 18). Perturbations of these processes have been proposed to underlie distinct physiological dysfunctions in PD-vulnerable neurons (19). Nonetheless, since the first introduction of Levodopa in the 1950s and the development of deep-brain stimulation in the 1990s, increased understanding of PD pathophysiology has not yet permitted the discovery of disease-modifying therapies.

As stated previously, PD is more than just a disease of DA and the SNc. Non-motor symptoms – including a reduced sense of smell, constipation, orthostatic hypotension, sleep disturbances, depression,

and anxiety – are likely to be due to impaired function and/or loss of non-DA neurons. (20) There has thus been a growing interest in better understanding the implications of other regions of the CNS and PNS in the progression of PD pathology. In the early 2000s, pioneering work by Braak and colleagues defined stages in PD based on the appearance of LP in various regions of the nervous system, correlating their findings to the symptomatic progression of the disease (2, 21, 22). Most notably, LP was detected in the dorsal IX/X motor nuclei, the intermediate reticular zone, the medulla oblongata, the pontine tegmentum, the caudal RN, the gigantocellular reticular nucleus, the coeruleus–subcoeruleus complex, the pars compacta of the substantia nigra, the basal prosencephalon, the mesocortex and the neocortex. However, multiple lines of evidence suggest that LP is not systematically seen in the PD brain and LP is also documented in healthy individuals (23). Also, in some cases of PD, and most notably in early-onset genetic forms, loss of SNc DA neurons has been reported to occur in the absence of detectable LP (24-26).

Although the role of LP in the pathogenesis of PD has been the subject of much debate (27), the detection of LP has remained central in investigations of the key brain regions and circuits underlying PD pathophysiology. In this context, it may be useful to focus attention on brain and PNS regions that show documented cell death and/or axonal degeneration, irrespective of the presence or absence of LP. This could perhaps provide new perspectives on the actual, more proximate, causes of the major symptoms of the disease and their progression. Relevant to the present point, in their most recent and insightful work, Braak and Tredici write, “We ascribed the same weight to axonopathy and nerve cell dysfunction (presumably attributable, but not limited, to the presence of Lewy pathology) as to neuronal death because the development of pathology together with neurotransmitter loss, axonal, and somatodendritic dysfunction in multiple neuronal populations could prove to be more stressful for involved neurons over time than premature cell death within a select neuronal population” (9).

11.05 Where and when does neuronal loss appear in pd?

Loss of neurons in the brain is thought to occur in the context of normal ageing. For example, there have been multiple publications reporting significant age-dependent decline in neuron number in the SNc (28-36), as well as in regions such as the PPN (37) and LC (38, 39). Above and beyond such cell loss associated with normal ageing, a key question is where in the brain can one find substantial neuronal loss in PD?

Although numerous publications have referred to cell loss occurring in many CNS and PNS regions in the context of PD, we believed it germane to re-evaluate the published scientific literature addressing this question.

To do so, we took great care to find work concentrating on neuronal loss and not only denervation (as is common for the heart, for example (40-42)). We found 90 primary research articles reporting PD-specific cell loss in the following regions (Table 4): the SNc, VTA, amygdala, cortex, DMV, hypothalamus, laterodorsal tegmental nucleus, LC, NBM, OB, oral pontine reticular nucleus, PPN, pre-supplementary motor cortex, RN, supraoptic nucleus, sympathetic/parasympathetic ganglia, and thalamus. These original articles span from 1953 to 2015. The techniques used to quantify cell loss varied, and we have classified them accordingly. Across all regions examined, 14 of the examinations were defined as observational, 39 as implicating manual counting, 18 used computer-assisted counting, and 26 used stereological counting methods. While informative, the value of observational studies can be considered limited given their lack of precision and the fact that they are greatly influenced by the observer. Lack of bias is also difficult to assure in studies involving manual counting. This technique is also unable to assure that a cell is not being counted twice if present in two subsequent sections. Other techniques such as computer-assisted counting were developed to improve on these aforementioned methods, however, these are also limited in that they often lack rigorous systematic sampling, are sensitive to tissue shrinkage, and are often unable to account for local tissue thickness, or for cells damaged on slice edges. These issues are systematically addressed using modern stereological counting techniques. Another issue to consider is that many of the studies included in this review, including those employing stereology, either did not use age-matched controls or did not state whether counting was conducted blind to diagnosis. Yet another apparent feature of this literature is the diversity of method iterations used, the varying number of brain regions assessed in each study and, importantly, the stage or type of PD studied (and how this was defined). Here, we will discuss the evidence of cell loss (if not otherwise stated, relative to healthy control cases), ordering the regions in subsections according to the strength of the evidence (Table 4).

11.06 Methodology and scales of PD progression

We searched the scientific literature using the search engines and databases of PubMed, Google Scholar and ScienceDirect. The following search terms were used: ‘PD’, and ‘cell loss’, ‘cell death’ or ‘reduced cell/neuron number’. Furthermore, these terms were used in combination with brain structure keywords: ‘SNc’, ‘VTA’, ‘LC’, ‘Raphe’, ‘DMV’, ‘PPN’, ‘NBM’ and ‘enteric system’ (‘ENS’), and ‘gut’. Review and original article abstracts were screened, then, where appropriate, read. Where any direct or indirect claim for cell loss was found (rather than only the presence of LP), the claim was followed to its original source. The Hoehn and Yahr scale (H&Y) is a widely used clinical rating scale, which defines broad categories of motor function in PD (where 1 is the least severe, and 5, most severe symptoms) (1).

11.07 Substantia nigra pars compacta

Loss of SNc DA neurons in PD is indisputable. Here we found 38 studies addressing this directly with a total of 612 brains. However, if we consider the methods used, we found that 10 of these studies were observational, 8 involved manual counting methods, 8 used computer-assisted methods, and 12 used stereology. Considering stereological methods as best practice for unbiased evaluation of cell number, 181 brains were quantified as such for SNc: still a large number. The average cell loss reported for studies involving stereological methods is ~ 68%. The definition and clinical stage of PD in most studies varied greatly, especially in reporting. For example, for the 12 studies using stereological methods, three papers (43-45) staged each case according to the Braak staging (to be expected given that Braak staging only came about in the early 2000s). In the same 12 studies, the age 'since disease onset' varied between 1 to 27 years when stated, the Hoehn and Yahr ratings (H&Y, used to describe the progression severity of PD symptoms) varied between 2 to 5 and the UPDSR score (that includes H&Y rating, symptoms and quality-of-life scores) was also on occasion provided. A correlation with disease duration/severity was found in 10 studies. It is relevant here to mention that some authors, including Gibb et al. (46) have discussed the selective vulnerability of restricted sub-regions within the SNc. These data are important and relevant to the progression of the field; however, we found this distinction absent in the majority of the work we examined.

11.08 Pedunculopontine nucleus and Locus coeruleus

The evidence for cell loss for both the PPN (11 studies), containing cholinergic neurons and the LC (18 studies), containing noradrenergic neurons, is also relatively strong.

For the PPN, four studies used stereological methods. In these four studies the average loss of cholinergic PPN neurons was 41% and the range of PD stages amongst the subjects evaluated was broad. For example, in Rinne, 2008 (47), the PD cases ranged from a H&Y rating of 2.5 to 5; in Karachi, 2010 (48), UDPRS score was used, and in both Hepp, 2013 (49) and Pienaar, 2013 (50), the PD cases were between Braak stages 4 and 6 and between 2 and 4, respectively. Although sample sizes were relatively small in these two studies, nine and eight, respectively, it is somewhat surprising that in the most advanced PD group, loss of cholinergic PPN neurons was not higher than for less advanced PD subjects, contrarily to the report by Rinne, 2008 (47).

Surprisingly, we found no study quantifying loss of LC neurons using stereological counting methods. For the LC, 221 brains were studied, with cell loss ranging from 'some' to 94%. Five of the studies were based on observational quantifications, 4 on manual counting and 9 used computer-assisted counting. In these 18 papers, when stated, the H&Y score was between 3 and 5, and disease duration was between 1

and 31 years. A correlation of the extent of cell loss with disease duration was found in two of these studies (51, 52).

11.09 Dorsal motor nucleus of the vagus, Raphe nuclei, nucleus basalis of Meynert and ventral tegmental area

Substantial cell loss has been documented in the DMV, containing cholinergic neurons, with 7 studies evaluating this loss in 49 cases. Of these, only one study (53) used stereology, where they reported 55% neuronal loss in eight PD cases, ranging from 5 to 24 years post diagnosis and reported correlation with disease duration/severity.

The importance of re-evaluating cell loss is PD is apparent when considering the serotonergic RN. For these nuclei, which are considered by many authors to be lost in PD, we found 7 papers describing neuronal loss varying between 0 to 90%. Cheshire et al. however, using stereology in 44 late-stage PD subjects, found no cell loss in the dorsal raphe nucleus (54). In the NBM, containing cholinergic neurons, we found 13 papers, 12 using manual counting methods and one observational, which estimated an average neuronal loss of between 'some' to 72%. No correlation with disease duration was reported. Surprisingly, only 8 studies directly evaluated neuronal loss in the VTA, a dopaminergic region often considered to be only modestly affected in PD. Of these, one study used stereology (55) to evaluate the loss of neurons in 3 cases of PD (or 6 including PD with a secondary diagnosis) that were between 1 and 27 years post diagnosis and reported an average neuronal loss of 31%. One paper reported correlation of the extent of cell loss with disease duration (56).

11.10 Thalamus, hypothalamus, olfactory bulb

Four studies reported neuronal loss in thalamic nuclei, with 2 using stereology (57, 58). In Henderson, 2000, 9 subjects with H&Y disease ratings between 2 and 5 statistically significant loss of 30 to 40% was reported in the centromedian-parafasicular complex. However, no loss was found in the motor thalamus in 9 subjects with similar H&Y disease ratings in the work of Halliday, 2005. Neuronal loss has also sometimes been reported in the hypothalamus (9 studies), with one using stereology; Thannickal et al. (59) reported a 50% cell loss in 10 PD cases, with increased loss with disease severity. Olfactory dysfunction is now well established as an early symptom of PD. Four studies evaluating cell loss in the olfactory bulb were reported. One of these (60) described a 57% decrease in neuronal number (identified as cells with "a prominent nucleolus surrounded by Nissl substance"), while the others (61-63), using stereology, reported a 100% increase in the number of TH-positive neurons.

11.11 Peripheral nervous system, spinal cord and other brain regions

Though there is substantial evidence for LP occurring in the ENS (64), we did not find any study reporting direct – quantitative evidence – for neuronal loss in the gut. Though it has been inferred that ENS glial cell loss is occurring (65), there is evidence that neuronal loss in the gut is not associated with PD (66). Of note, a publication often cited in support of neuronal loss in the ENS (67) shows, in fact, neuronal loss in the DMV. With regards to the spinal cord, published evidence is also scarce; of the studies most relevant here, Wakabayashi et al. (68), using manual counting methods, described a loss of 31% and 43% respectively in the 2nd and 9th thoracic segments of the intermediolateral of the spinal cord. For the amygdala, the pre-supplementary motor cortex, several other cortical regions, the laterodorsal tegmental nucleus and the oral pontine reticular nucleus, we found only single studies supporting loss, with stereology used for the amygdala (30% loss) (69) and cortex (10% loss) (69) (see Table 4).

11.12 Regional order of cell loss?

In summary, it seems clear that there is some level of cell loss in PD in restricted regions including the SNc, LC, NBM, PPN, DMV, VTA and probably the RN. However – because of the lack of data for some regions, the variety of techniques used to count neurons, potentially numerous unintentional sources of bias, and because of the inconsistency in criteria used for subject sampling – firm conclusions are somewhat limited. In particular, it is difficult to conclude on the relative extent and temporal order of cell loss in these different brain regions as a function of disease progression, information that would be critical to advance the field. Indeed, a direct comparison of the extent of neuronal loss in different regions examined in different studies is hazardous, even if stereological studies were to be selected. Interestingly, of the 38 studies we identified evaluating cell loss in the SNc, only 5 of these also looked at the VTA, and of these only 1 used stereology. Given the importance of the difference in vulnerability of these two nuclei, a systematic evaluation of the extent of loss of these neurons in PD would be very informative. But even if as a technique, stereology mitigates for most of the classic biases, it is still unable to account for the variation in subject sampling, i.e. variation in disease duration, sex and age, unless these criteria were considered in a similar way for each study. Unfortunately, this has not, thus far, been the case. In conclusion, it seams clear that stereological studies comparing multiple regions in the same subjects and these regions in subject at different stages of PD are critically needed to advance the field.

11.13 Table of 90 studies quantifying the loss of neurons in the brain in PD

Publications	Technique	N (ctrl)	Loss of neurons (%)	Comparison group info (healthy controls unless stated otherwise)	Blinded / age matched Yes — Y Not stated — NS	Stated diagnosis, scale of severity, disease duration (expressed in range or mean, when available)	Other regions counted	Correlations (with disease severity, duration or age)
Substantia Nigra pars Compacta (SNc)								
Greenfield, 1953 (144)	o	19 (22)	some	--	NS	iPA, <1-20 years	LC	--
Pakkenberg, 1965 (145)	m	10 (10)	66	Healthy controls and two young controls	NS/Y	iPA	--	--
Bernheimer, 1973 (146)	o	69 (0)	some	No healthy controls, compared to type of PD and Huntington's disease	NS/Y	PD, H&Y, 1-47 years	--	--
Rajput, 1976 (147)	o	6 (1)	some	--	NS	iPA, H&Y, 3-18 years	LC, DMV, Cortex, Hypothalamus, Intermediolateral spinal cord, sympathetic ganglia	--
Gaspar, 1984 (148)	o	32 (6)	some	--	Y/Y	iPD, 2-23 years	LC, NBM	--
Tagliavini, 1984 (149)	o	6 (5)	some	--	NS/Y	iPD, 5-13 years	NBM	--
Chan-Palay, 1988 (150)	o	9 (22)	some	--	Y/NS	PD	NBM	--
Gibb, 1988 (151)	m	34 (-)	-	No healthy controls, compared young and old onset	NS	PD, 1-34 years	--	--
Hirsch, 1988 (70)	c	4 (3)	77	--	NS	PD	A10, A8, CGS	--
German, 1989 (71)	c	5 (3)	61	--	NS/Y	PD, 5-27 years	VTA	--
Rinne, 1989 (152)	s	12 (18)	60	--	NS/Y	iPD, H&Y II-V	--	Y
Zweig, 1989 (153)	o	6 (8)	Mild to severe	Not compared - estimation	NS/Y	PD, 5-14 years	PPN, DR, NBM	--
Gibb, 1990 (46)	m	6 (6)	75	--	NS	PD	--	--
Halliday, 1990 (72)	c	4 (4)	68	--	NS/Y	PD	SNc + LC, RN, PPN, DMV	Y (dementia score)
Fearnley, 1991 (30)	m	20 (36)	20-90	--	NS/Y	PD, 1.5-38 years	--	Y (also in controls)
Pakkenberg, 1991 (154)	s	7 (7)	66	--	NS/Y	PD, 4-16 years	--	--
Paulus, 1991 (73)	m	39 (14)	59	--	NS/Y	PD, H&Y III-V, 1-31 years	LC, DRN, NBM	--

Xuereb, 1991 (155)	o	5 (5)	some	--	NS/Y	PD	Thalamus (multiple nuclei)	--
Moller, 1992 (156)	c	3 (3)	80	--	NS/Y	PD	--	--
Zweig, 1993 (157)	m	13 (14)	some	--	Y	PD, H&Y 4.5, 11 years	LC, VTA, NBM	--
Mouatt-Prigent, 1994 (158)	c	4 (3)	76	--	NS/Y	iPD	VTA	--
Ma, 1995 (159)	s	4 (7)	70	--	NS	PD	--	--
Halliday, 1996 (160)	s	11 (15)	37-75	--	NS/Y	PD, 1-18 years	--	Y
Ma, 1996 (161)	c	20 (8)	76	--	NS/Y	PD	--	--
Ma, 1997 (162)	s	12 (12)	55	--	NS/Y	PD, H&Y III-V, 3-17 years	--	Y
Damier, 1999 (56)	c	5 (5)	86	--	NS	iPD	VTA	Y
Henderson, 2000 (57)	c	9 (8)	69	--	NS/Y	PD, H&Y II-V, 3-17 years	Centromedian–Parafascicular Complex, mediodorsal or anterior principal nucleus	--
Zarrow, 2003 (74)	m	19 (13)	78	Healthy controls, AD	NS/Y	iPD, 12.4 years	LC, NBM	
Greffard, 2006 (163)	o	14 (5)	50	--	NS/Y	iPD, UPDRS3 = 53, 8.5 years	--	Y
Rudow, 2008 (34)	s	8 (23)	~80 vs young, ~75 vs old controls	Young, middle aged and old healthy controls	NS/Y	PD, 7-20 years	--.	Y, in controls
Beach, 2009 (164)	o	66 (87)	some	Healthy controls, ILDB, DLB, ADLB, ADNLB	Y/NS	PD + DLB, UPDRS = 41, 10.6 years	--	--
Karachi, 2010 (48)	s	12 (8)	69-88	--	Y	PD, UPDRS	PPN	--
Milber, 2012 (45)	s	13 (17)	70	Healthy controls, iLBD	Y/NS	PD, Braak stage I-VI, 8.3 years.	--	Y in iLBD
Kordower, 2013 (165)	s	28 (9)	50-90	--	Y	PD, 1-27 years	--	Y
Dijkstra, 2014 (43)	s	24 (12)	56	Healthy controls, iLBD	Y	PD and iLBD, Braak stage 0-VI, H&Y, 13.6 years	--	Y
Kraemmer, 2014 (166)	m	4 (0)	-	No healthy controls, compare to AD, CJD, CBS, NPH	Y/NS	PD and DLB, 2-4 years	--	--

Cheshire, 2015 (54)	s	44 (17)	75	--	Y	PD, LID severity, 14.8 years	RN	--
Iacono, 2015 (44)	s	6 (6)	82	--	Y	iPD and iLDB, Braak stage I-IV, H&Y 2-5,	--	--
38 o10, m8, c8, s12		612(452)						
Locus Coeruleus (LC)								
Rajput, 1976 (147)	o	6(1)	some	--	NS	iPA H&Y, 3-18 years	SN, DMV, Cortex, Hypothalamus, Intermediolateral spinal cord, sympathetic ganglia	--
Gaspar, 1984 (148)	o	32 (6)	some	--	Y	iPD, 2-23 years	SNc, NBM	--
Hirsch, 1988 (70)	c	4 (3)	55	--	NS	PD	SNc, A10, A8	--
Chan-Palay, 1989 (167)	c	6 (3)	31-94*	--	NS/Y	PD	--	--
Zweig, 1989 (153)	o	6 (8)	Mild to severe	Not compared - estimation	NS/Y	PD, 5-14 years	PPN, SNc, DR, NBM	--
Halliday, 1990 (72)	c	4 (4)	68	--	NS/Y	PD	SNc + LC, RN, PPN, DMV	--
Gai, 1991 (51)	c	6 (5)	74	--	NS/Y	iPD, 5-30 years	PPN, LTN, OPN, RN	Y
Paulus, 1991 (73)	m	37 (12)	63	--	NS/Y	PD, H&Y III-V, 1-31 years	SNc, DRN, NBM	--
German, 1992 (168)	c	6 (7)	21-93	Healthy controls, AD, down-syndrome	NS/Y	PD, 5-16 years	--	--
Patt, 1993 (169)	o	8 (8)	some	--	NS	PD	--	--
Zweig, 1993 (157)	m	13 (14)	46-69	--	Y/Y	PD, H&Y 4.5, 11 years	SNc, VTA, NBM	--
Hoogendijk, 1995 (170)	c	5 (5)	39 NS	Healthy controls, AD, ALS	NS/Y	PD, 7 years	--	--
Bertrand, 1997 (52)	c	11 (6)	58-78	--	NS	PD	--	Y
Zarrow, 2003 (74)	m	19 (13)	83	Healthy controls, AD	NS/Y	iPD, 12.4 years	SNc, NBM	--
Brunnstom, 2011 (171)	m	25(0)	mild-severe	Healthy controls, AD	Y/NS	DLB and PD dementia	--	--
Mc Millan, 2011 (172)	m	7 (8)	71-88	Healthy controls, AD, DLB	Y	PD, 7-25 years	--	--
Dugger, 2012 (173)	c	21 (11)	some	--	NS/Y	LBD, 8.4 years	PPN	--

Del Tredici, 2013 (174)	o	5 (1)	some	--	NS	PD, H&Y 3-5, 7-15 years	--	--
18 o5, m4, c9, s0		221(115)						--
*31 w/o dementia, 48 w/ dementia, 94 if Non-responsive to L-dopa								
Nucleus Basalis of Meynert (NBM)								
Arendt, 1983 (175)	m	5 (14)	70	--	NS/Y	Postencephalitic PD	--	--
Candy, 1983 (176)	m	5 (5)	some	Healthy controls, AD	NS	PD	--	--
NakaNo, 1983 (177)	m	2 (5)	90	--	NS/Y	PD-dementia complex of Guam, 4-5 years	--	--
Whitehouse, 1983 (178)	m	12 (10)	45-71	--	Y	iPD, 4-26 years	--	--
Gaspar, 1984 (148)	m	32 (6)	36	--	Y	iPD, 2-23 years	SNc, LC	--
NakaNo, 1984 (179)	m	11 (13)	60	--	NS/Y	PD, 1-17 years	--	--
Tagliavini, 1984 (149)	m	6 (5)	46-69	--	NS/Y	iPD, 5-13 years	SNc	--
Perry, 1985 (180)	m	4 (8)	17-72	Healthy controls, AD	NS/Y	PD	--	--
Rogers, 1985 (181)	m	4(5)	some	Healthy controls, PSP, Creutzfeldt-Jakob disease, ALS, MS and AD (+ individual cases of other diseases)	NS/Y	PD	--	--
Chan-Palay, 1988 (150)	m	9 (22)	~50	Healthy controls, AD	Y/NS	PD	SNc	--
Paulus, 1991 (73)	m	40 (17)	some	--	NS/Y	PD, H&Y III-V, 1-31 years	SNc, LC, DRN	--
Zweig, 1993 (157)	o	13 (14)	some	--	Y	PD, H&Y 4.5, 11 years	LC, SNc, VTA	--
Zarrow, 2003 (74)	m	19 (13)	37	Healthy controls, AD	NS/Y	iPD, 12.4 years	SNc, LC	--
13 o1, m12, c0, s0		162(137)						
Pedunculopontine Nucleus (PPN)								
Hirsch, 1987 (182)	c	6 (4)	57	Healthy controls, supranuclear palsy	NS	PD	--	--
Jellinger, 1988 (183)	m	14 (15)	53	--	NS/Y	PD, 10 years	--	--
Zweig, 1989 (153)	m	4 (8)	46-69	--	NS/Y	PD, 10-14 years	--	--
Halliday, 1990 (72)	c	4 (4)	57	--	NS/Y	PD	SNc + LC, RN, DMV	--

Gai, 1991 (51)	c	6 (5)	43	--	NS/Y	iPD, 5-30 years	LTN, OPN, RN, LC	Y
Rinne, 2008 (47)	s	11 (9)	40	--	NS/Y	PD, H&Y 2.5 and 5, 9.3 years	--	Y
Schmeichel, 2008 (184)	m	13 (11)	65	Healthy controls, MSA	Y/NS	DLB, 3-16 years	Laterodorsal tegmental nucleus	--
Karachi, 2010 (48)	s	12 (8)	31-38	--	Y	PD, UPDRS 0-IV	SN	--
Dugger, 2012 (173)	c	21 (11)	some	--	NS/Y	LBD, 8.4 years	LC	--
Hepp, 2013 (49)	s	9 (9)	41	Healthy controls, DLB	Y	PD, Braak stage IV-VI, H&Y IV-V, 8-26 years	--	--
Pienaar, 2013 (185)	s	8 (5)	50	--	Y	PD, Braak stage II-IV, 6-13 years	--	--
11 o0, m3, c4, s4		108 (89)						
Hypothalamus								
Rajput, 1976 (147)	o	6 (1)	None	--	NS	iPA, H&Y, 3-18 years	SN, LC, DMV, Cortex, intermediolateral spinal cord, sympathetic ganglia	--
Kremer, 1992 (186)	m	8 (15)	None	--	NS	PD	--	--
Kremer, 1993 (187)	m	8 (7)	None	--	NS/Y	iPD, 4-17 years	--	--
Purba, 1994 (188)	m	6 (6)	20	--	NS/Y	PD	--	--
Nakamura, 1996 (189)	m	8 (6)	None	--	NS/Y	iPD	--	--
Ansorge, 1997 (190)	m	7 (8)	29-Dec	--	NS/Y	PD, 18 years	--	--
Hoogendijk, 1998 (191)	m	12 (6)	None	--	Y	iPD	--	--
Fronczek, 2007 (192)	c	9 (9)	45	--	Y	PD, late-stage	--	--
Thannickal, 2007 (59)	s	10 (5)	50	--	NS/Y	PD, H&Y I-V, 4-23 years	--	Y
9 o1, m6, c1, s1		74(63)						
Dorsal Motor Nucleus of the Vagus Nerve (DMV)								
Eadie, 1963 (193)	m	8(5)	30	--	NS/Y	PD	Hypoglossal nuclei, nucleus ambiguus	--
Rajput, 1976 (147)	o	6(1)	some	--	NS	iPA, H&Y, 3-18 years	SN, LC, Cortex, Hypothalamus,	--

							Intermediolateral spinal cord, sympathetic ganglia	
Halliday, 1990 (75)	c	4 (4)	77	--	NS	PD	RN	--
Halliday, 1990 (72)	c	4 (4)	77	--	NS/Y	PD	SNc + LC, RN, PPN	--
Saper, 1991 (194)	m	5(5)	60	--	NS	PD, 2-16 years	--	--
Gai, 1992 (53)	s	8(6)	55	--	NS/Y	PD, 5-24 years	Hypoglossal nucleus	Y
Benarroch, 2006 (67)	o	14(12)	50	--	Y/NS	PD or LBD, 10 years	Nucleus ambiguus	--
7 o2, m2, c2, s1		49(37)						
Raphe Nuclei (RN)								
Yamamoto, 1985 (195)	m	2 (1)	50-90	--	NS/Y	iPD	--	--
Halliday, 1990 (75)	c	4 (4)	0 dorsal-56 median	--	NS	PD	DMV	--
Halliday, 1990 (72)	c	4 (4)	0 dorsal-44 obscurus -60 median	--	NS/Y	PD	SNc + LC, PPN, DMV	--
Gai, 1991 (51)	c	6 (5)	76	--	NS/Y	iPD, 5-30 years	PPN, LTN, OPN, LC	--
Paulus, 1991 (73)	m	23 (6)	37	--	NS/Y	PD, H&Y III-V, 1-31 years	SNc, LC, RN, NBM	--
Benarroch, 2007 (196)	m	14 (12)	60-67	--	Y	DLB, 5-20 years	--	--
Cheshire, 2015 (54)	s	44 (17)	None	--	Y	PD, LID severity, 14.8 years	SNc	--
7 o0, m3, c3, s1		97 (49)						
Ventral Tegmental Area (VTA)								
Javoy-Agid, 1984 (197)	m	2 (2)	77	--	NS	PD	--	--
Hirsch, 1988 (70)	c	4 (3)	48	--	NS	PD	SNc, A10, A8, CGS	--
German, 1989 (71)	c	5 (3)	42	--	NS/Y	PD, 5-27 years	SNc	--
Zweig, 1993 (157)	m	13 (14)	some	--	Y	PD, H&Y 4.5, 11 years	LC, SNc, NBM	--

Mouatt-Prigent, 1994 (158)	c	4 (3)	some	--	NS/Y	iPD	SNc	--
Dymecki, 1996 (198)	m	7 (6)	41-62	--	NS/Y	PD, long-term	--	--
McRitchie, 1997 (55)	s	3 (3)	31	--	NS/Y	iPD, 1-27 years	A8, A10	--
Damier, 1999 (56)	c	5 (5)	46	--	NS	iPD	SNc	Y
8		43 (39)						
o0, m3, c4, s1								
Olfactory Bulb (OB)								
Pearce, 1995 (60)	m	7 (7)	57	--	NS/Y	PD, 8-19 years	--	--
Huisman, 2004 (61)	s	10 (10)	increase of 100	--	NS/Y	PD, 4-23 years	--	--
Huisman, 2008 (62)	s	20 (19)	increase of 100 in female	--	Y	iPD, 3-30 years	--	--
MundinaNo, 2011 (63)	s	6 (15)	increase of 100	--	NS/Y	PD, Braak stage II-V	--	--
4		43 (51)						
o0, m1, c0, s3								
Thalamus								
Xuereb, 1991 (155)	m	5 (5)	None	--	NS/Y	PD	Thalamus (multiple nuclei)	--
Henderson, 2000 (199)	c	9 (10)	40-55	--	NS/Y	PD, H&Y II-V, 7.2 years	Caudal intralaminar nuclei, limbic thalamic nuclei	--
Henderson, 2000 (57)	s	9 (8)	50-70	--	NS/Y	PD, H&Y II-V, 3-17 years	SNc, Centromedian–parafascicular complex, mediodorsal or anterior principal nucleus	--
Halliday, 2005 (58)	s	9 (9)	None	--	NS/Y	PD, H&Y II-V, 9 years	Motor thalamus, Cortex	--
4		32(32)						
o0, m1, c1, s2								
Sympathic/parasympathic ganglia								
Rajput, 1976 (147)	o	6 (1)	some	--	NS	iPA, H&Y, 3-18 years	SN, LC, DMV, Cortex, Hypothalamus	--

Wakabayashi, 1997 (68)	m	25 (25)	31-43	--	NS/Y	PD	--	--
Benarroch, 2006 (67)	o	14 (12)	None	--	Y/NS	PD or LBD, 10 years	DMV, nucleus ambiguus	--
3 o2, m1, c0, s0		45 (38)						
Cortex								
Rajput, 1976 (147)	o	6 (1)	None	--	NS	iPA, H&Y, 3-18 years	SN, LC, DMV, Hypothalamus, Intermediolateral spinal cord, sympathetic ganglia	--
Pedersen, 2005 (200)	s	10 (12)	None	--	NS/Y	PD, 2-25 years	--	--
2 o1, m0, c0, s1		16 (13)						
Pre-supplementary and premotor cortex								
MacDonald, 2002 (201)	m	5 (5)	32-45	--	Y	PD, 10-17 years	--	--
Halliday, 2005 (58)	s	9 (9)	None	--	NS/Y	PD, H&Y II-V, 9 years	Motor thalamus	--
2 o0, m1, c0, s1		14 (14)						
Amygdala, corticomedial complex								
Harding, 2002 (69)	s	18 (16)	30	--	Y	PD, 13 years	--	--
Hippocampus								
Joelving, 2006 (202)	s	8 (8)	None	--	NS/Y	PD, 2-25 years	--	--
Laterodorsal tegmental Nucleus (LTN)								
Gai, 1991 (51)	c	6 (5)	41	--	NS/Y	iPD, 5-30 years	PPN, OPN, RN, LC	Y
Oral pontine reticular Nucleus (OPN)								
Gai, 1991 (51)	c	6 (5)	41	--	NS/Y	iPD, 5-30 years	PPN, LTN, RN, LC	Y

Table 4 List of 90 studies quantifying the loss of neurons in the brain in PD

Included in the table are the technique used for quantification (o, observation; m, manual c, computer assisted; s, stereological counting), the number of subjects and controls (ctrl) studied, the estimated % loss of neurons, any particularity in the comparison group, mention if studies were performed blind and with age-matched controls, the stated diagnosis, scale of severity and

disease duration when mentioned and note on other regions counted. Where an average value of loss was not given by authors, this number was calculated from available data.

ABREVIATIONS: AD, Alzheimer Disease; ADLB, Alzheimer's Disease with Lewy bodies; ADNLB, Alzheimer's Disease with no Lewy bodies; ALS, Amyotrophic Lateral Sclerosis; CBS, corticobasal syndrome; CGS, central grey substance; CJD, Creutzfeldt-Jakob disease; ctrl, control; DLB, dementia with Lewy bodies; H&Y, Hoehn and Yahr scale; iPA, idiopathic paralysis agitans; LBD or iLBD, Lewy body disease or idiopathic Lewy body disease; LDB or iLDB, dementia with Lewy bodies or idiopathic dementia with Lewy bodies; LID, levodopa (L-dopa)–induced dyskinesias; MS, multiple sclerosis; MSA, multiple system atrophy; NPH, normal pressure hydrocephalus; PD or iPD, Parkinson’s Disease or idiopathic Parkinson’s Disease; PSP, progressive supranuclear palsy; UPDRS, unified Parkinson Disease rating scale.

11.14 What are the common features shared by neurons affected in pd?

Although, as mentioned previously, the evidence for the extent of cell loss in regions other than the SNc in the PD brain is not always sufficiently documented, it is clear that some level of cell loss occurs in a limited subset of regions beyond the SNc (Figure 20) or, to the least, that neuronal functions including neurotransmission are perturbed in multiple neuronal circuits. It is therefore of great interest to identify some of the biological features that distinguish neuronal subgroups in terms of their basal vulnerability to some of the cellular stresses that are invoked to trigger PD, including altered proteostasis (due to lysosomal and/or proteosomal impairment), mitochondrial dysfunction and sustained oxidant stress (including from highly reactive DA metabolites).

Several groups have been tackling this question by interrogating the characteristics that render neurons, starting with those of the SNc, particularly vulnerable to degeneration / cell death (76-78). It is likely that some shared functional or structural properties are responsible for selective vulnerability of affected nuclei, as opposed to features truly unique to SNc DA neurons. The causative characteristic(s) should be present in all affected neurons, but also be absent in neurons that do not degenerate or that degenerate much later in the disease. Four main converging hypotheses on selective vulnerability in PD have been gaining attention lately (Figure 20 B), related to DA toxicity, iron-content, autonomous pacemaking and axonal arborization size. The next section will explore the likelihood that these hypotheses can explain why select neuronal populations are particularly vulnerable in PD.

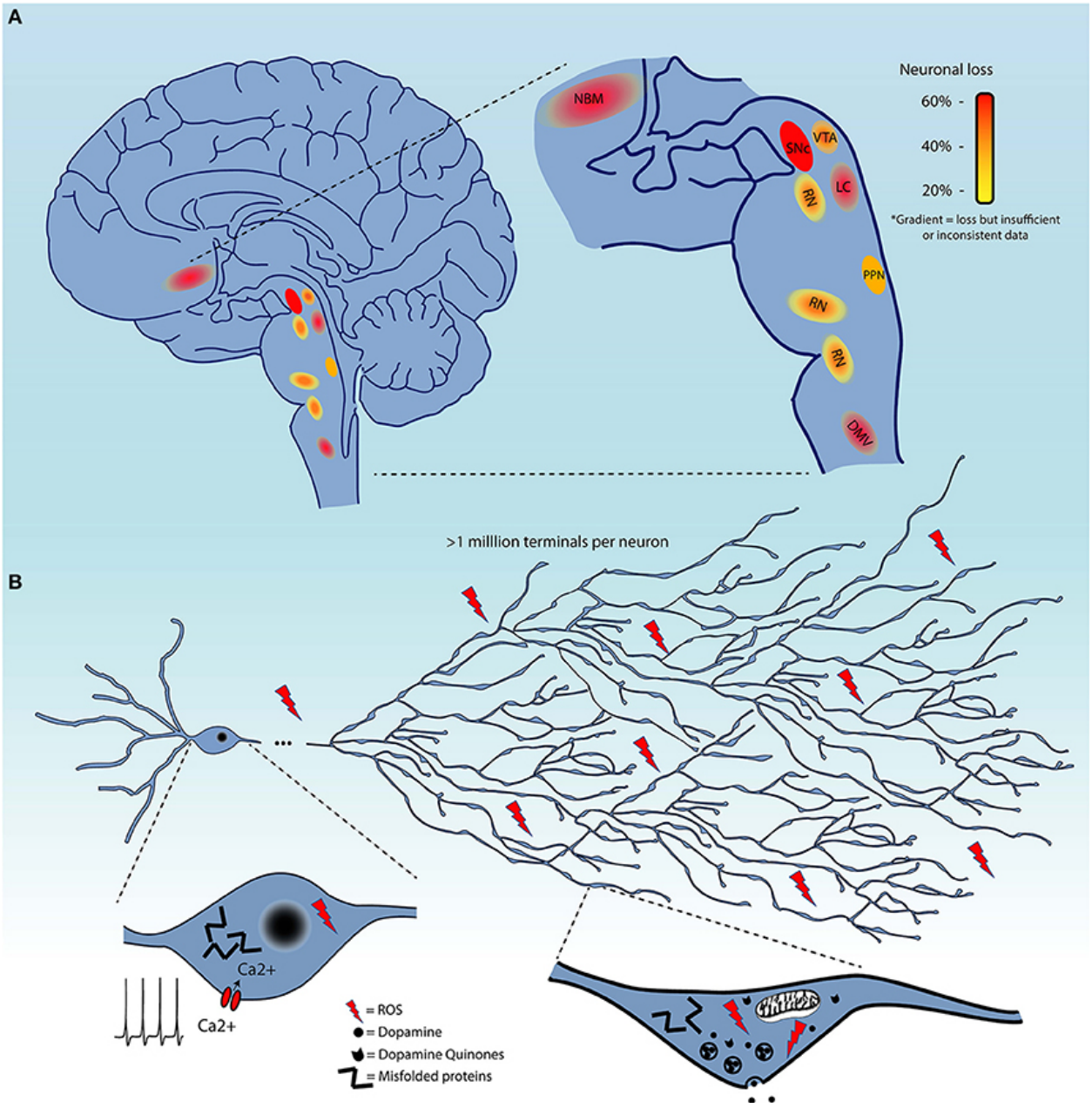


Figure 20 Evidence for cell loss and selective vulnerability in PD: a critical review

A. Schematic representation of brain regions hypothesized to demonstrate cell loss in Parkinson's disease, color-coded as a function of the weight of the evidence supporting the possibility of actual cell loss, and not only cellular dysfunction and Lewy pathology. Neuronal loss: red = 60%, orange = 40% and yellow = 20%. Color gradient indicates a range of possible loss, due to insufficient or inconsistent data. **B**. Summary of converging hypotheses regarding the origin of the selective vulnerability of neurons in the context of Parkinson's disease. Whether due to the exceptionally large axonal arbor of affected neurons or their electrophysiological properties, including calcium-dependent pacemaking, high levels of oxidant stress in the somatodendritic and axonal domain is thought to play a key role in cellular dysfunction and cell loss. Pathological protein aggregation and reactive dopamine quinones are considered as additional precipitating factors.

11.15 Dopamine toxicity

Firstly, it has been suggested that DA neurons in general are most at risk because they produce DA as a neurotransmitter, a molecule that can be toxic in certain conditions through the generation of reactive quinones during its oxidation (79). This oxidation has been proposed to be implicated in the production of neuromelanin in SNc DA neurons. These DA quinones have been shown to interact with and negatively impact the function of mitochondrial protein complexes I, III and V (80) and of other proteins such as tyrosine hydroxylase, the DA transporter and α-synuclein (81, 82). Such reactive by-products can promote mitochondrial dysfunction, pathological aggregation of proteins such as α-synuclein and oxidative stress (83). Increasing the vesicular packaging of DA accordingly reduces the vulnerability of DA neurons, while down-regulating vesicular packaging has the opposite effect (84-87). Although highly relevant, this phenomenon alone does not readily explain the differential vulnerability of different dopaminergic neuron subgroups (such as SNc vs VTA) and cannot contribute to the potential vulnerability of non-dopaminergic neurons in PD. Also, in the context of DA-induced toxicity, it is puzzling that levodopa therapy, acting to increase DA synthesis, does not appear to accelerate cell loss (88, 89). For these reasons, even if DA toxicity most certainly contributes to degeneration of SNc DA neurons, it is certainly not the sole factor driving neuronal death in PD.

11.16 Iron content

Secondly, iron content is thought to also be an important contributor to the selective vulnerability of SNc DA neurons. Iron is known to be able to generate ROS by the Fenton reaction and has been shown to accumulate with age in SNc (90-92). Since the mitochondrial electron transport chain relies on iron sulphur clusters for its function and since it is believed that SNc neurons have particularly high bioenergetic demands (76, 78, 93), elevated iron content could in part underlie elevated and sustained mitochondrial activity. Another interesting feature of iron in SNc DA neurons is that it can be chelated by neuromelanin, which renders it unavailable for mitochondrial function. Even if the affinity of iron for neuromelanin is much lower than for other iron binding proteins such as ferritin, it is possible that accumulation of neuromelanin and loss of ferritin concentration with age impacts gradually mitochondrial function, which could eventually promote cell death. However, data about potential iron content and iron-binding protein concentration changes in PD is still a matter of debate (94, 95). In addition, data is lacking on iron levels in other brain regions presenting cell death in PD. In fact, the only other region studied in this context has been the LC, which did not show high iron relative to the SNc (96-99).

11.17 Autonomous pacemaking

A third highly attractive hypothesis to explain the vulnerability of SNc DA neurons has its origins in the fact that these neurons demonstrate autonomous pacemaking. Many receptors/channels can potentially modulate the excitability and survival of DA neurons (100). The fact that pacemaking activity in SNc DA neurons is accompanied by slow oscillations in intracellular calcium concentrations, caused by the opening of voltage-dependent Cav1 plasma membrane calcium channels (Cav1.1 and 1.3) has recently renewed interest to this topic. In the Cav1 family, Cav1.3 has been suggested to be of particular interest because its voltage-sensitivity and inactivation properties allow a subset of the calcium channels to always stay open during pacemaking, causing extensive calcium entry (77). These oscillations have a positive contribution to cell physiology because they help maintain pacemaking and directly promote mitochondrial oxidative phosphorylation (OXPHOS) (101). However, by doing so, they have been proposed to also promote chronically high levels of ROS production (102, 103). Along with a reduction in mitochondrial function with age, chronically elevated oxidative stress has been proposed to be a causative factor in the decline of neuronal survival (104). Although CaV currents and autonomous pacemaking are also a feature of LC and DMV neurons (102, 103), their influence on the survival of these neurons has not yet been investigated. The fact that other neuronal populations also expressing Cav1.3 such as hippocampal neurons (105) and striatal spiny projection neurons (106) do not degenerate in PD highlights the possibility that the particular vulnerability of SNc DA neurons is due to a combination of physiological phenotypes and not only intracellular calcium oscillations. Intriguingly, recent post-mortem studies showed that there was no decrease in Cav1.3 mRNA level in early or late stage PD in human SNc compared to controls (106, 107), despite significant loss of SNc neurons. Finally, in addition to CaV channels, ATP sensitive potassium channels (K-ATP) have also been reported to regulate the excitability and vulnerability of SNc DA neurons (108).

11.18 Axonal arborization size

A fourth hypothesis proposes that vulnerable neurons such as those of the SNc are particularly vulnerable because of the massive scale of their axonal arborization, leading to very high numbers of axon terminals, elevated energetic requirements and chronically high oxidant stress. Indeed, it has been shown that SNc DA neurons have an exuberant and highly arborized axonal arborization with estimates upwards of a million neurotransmitter release sites per SNc DA neuron in humans (76, 109): this would make them some of the most highly arborized neurons in the nervous system. This characteristic has the potential to place a very large bioenergetic burden on these cells, leaving little margin for additional bioenergetic stress (76, 78, 93). It has been calculated that the ATP requirement for propagation of one AP grows exponentially with the level of branching (110). In a recent publication (78), we demonstrated in vitro that reducing the

axonal arbor size of SNc DA neurons to a size more similar to that of VTA DA neurons using the axonal guidance factor Semaphorin 7A, was sufficient to greatly reduce basal OXPHOS and reduce their vulnerability to toxins including MPP+ and rotenone. Although as previously discussed, the extent of neuronal loss is still unclear for many neuronal populations, it does seem likely that most neuronal nuclei affected in PD include neurons that are relatively few in number, but all possess long and profuse unmyelinated axonal arbors and a large number of axonal terminals (111-116). However, comparative data evaluating axonal arbor size amongst these populations and in populations of neurons that do not degenerate in PD is presently lacking. An interesting case is that of striatal cholinergic interneurons, which were previously estimated in rats to present 500 000 axonal varicosities (117, 118), but have not been reported to degenerate in PD. This estimate was obtained by dividing the estimated number of terminals by the estimated number of cholinergic interneurons in the striatum, which was based on the total number of striatal neurons and the proportion of cholinergic interneurons. Considering recent stereological counting of the number of neurons in the rat striatum, it is possible that the total number of terminals estimated for striatal cholinergic neurons may have been overestimated by a factor of six (119). Careful quantitative and comparative studies are clearly needed.

11.19 A global bioenergetic failure hypothesis

One commonality between these four hypotheses is that they all suggest that vulnerable neurons are under intense mitochondrial / bioenergetic demand. This could alter the oxidative stress response by depleting antioxidants like glutathione (GSH), as previously suggested to occur in the PD brain (120-122). This stress could also, at a certain point, place the cells in a situation in which the rate of OXPHOS required to sustain neurotransmitter release and cellular excitability leaves too little of the cell's resources to sustain other key cellular functions such as degradation of damaged or misfolded proteins (77). This could lead to preferential dysregulation of axon terminals, triggering a dying back cascade culminating later in cell death (5, 123, 124). Approximately half of the oxygen consumed by mitochondria in SNc DA neurons appears to be used by activity-dependent cellular processes such as firing and neurotransmitter release (78). In this context, axon terminal degeneration seen early in the disease, prior to cell death, could be in part an attempt by stressed neurons to adapt to such excessively high metabolic needs. Such a dying back process could also lead to increased amounts of damaged axonal proteins to manage, potentially promoting their accumulation in intracellular inclusions. Since α-synuclein is highly concentrated in axon terminals, it is possible that retraction of axonal processes in a cell where protein degradation systems are overwhelmed promotes creation of pathological aggregates of this protein, thus accelerating cell death. Interestingly, lysosomal defects secondary to GBA1 gene mutations are present in up to 10% of PD patients. This gene

encodes a glucocerebrosidase responsible for breaking down lysosomal glucolipid. When GBA1 is mutated, the level of glucolipid and of misfolded proteins increases in neurons. This is likely to represent a particular challenge for highly arborized neurons such as SNc DA neurons, perhaps explaining why such mutations are now considered the greatest genetic risk factor for PD (125-131). Similarly, mutations in gene products implicated in mitophagy and mitochondrial antigen presentation (PARK2, PINK1) (132, 133), oxidative stress response (PARK7) (134, 135) or vesicular trafficking (LRRK2) (136, 137) are present in familial forms of PD and their detrimental impact on cellular functions could represent larger challenges for highly arborized and energetically ambitious neurons.

11.20 Towards better treatments of PD

In the context of the hypotheses discussed here regarding the origin of the selective vulnerability of neurons in PD, novel strategies to promote survival and preservation of cellular functions amongst challenged neuronal populations could possibly come from approaches that aim to reduce mitochondrial burden by either reducing neuronal metabolic needs or optimizing mitochondrial function. As an example, the CaV1.3 channel inhibitor isradipine is presently in phase 3 clinical trial and could possibly reduce the calcium- and activity-related metabolic stress of SNc DA neurons leading to neuroprotection (138). Other promising molecules could come from the repurposing of drugs used to treat diabetes and other metabolic diseases. One example is exenatide, a glucagon-like-peptide-1 agonist that has the property to increase glucose-induced insulin secretion, to prevent the rise of ROS and prevent decreases of mitochondrial function in diet-induced obese mice (139). This agonist was found to reduce the loss of DA neurons in the MPTP mouse model (140) and a recent clinical trial has shown improved motor function after 60 days of administration to PD patients (141). Overexpression of the mitochondrial deacetylase SIRT3 has also recently been shown in two studies to reduce basal OXPHOS by DA neurons and to protect SNc neurons in rodent models of PD (142, 143). With further discoveries of the underlying causes of the intrinsic vulnerability of neurons in the PD brain and PNS, multiple other strategies may soon be devised to address some of the specific challenges faced by energetically challenged neurons.

In conclusion, although the presently available data strongly argue that multiple populations of neurons are affected in PD and degenerate to varying extents, new work is needed to provide a more systematic, comparative, and time-dependent quantification of neuronal loss in this disease. More comprehensive and convincing data on cell death and axon terminal dysfunction in PD will likely provide additional impetus for new work aiming to solve the long-awaited challenge of identifying disease-modifying therapeutic approaches for this incapacitating and ill-treated disorder.

11.21 Acknowledgements

This work was supported by a grant from the Brain Canada and Krembil Foundations, as well as by the Canadian Institutes of Health Research. We would like to express our gratitude to Ms. Sarah Ouellet for her help preparing the illustrations and to colleagues who graciously provided feedback on an earlier version of this manuscript

11.22 References

1. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967;17(5):427-42.
2. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiology of aging. 2002;24(2):197-211.
3. Parkinson J. An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci. 2002;14(2):223-36; discussion 2.
4. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol. 2010;67(6):715-25.
5. Burke RE, O'Malley K. Axon degeneration in Parkinson's disease. Exp Neurol. 2013;246:72-83.
6. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2015;30(12):1591-601.
7. Lewy FHaEF. Paralysis agitans. in: Handbuch der Neurologie, hsg. von Max Lewandowsky, Dritter Band, Spezielle Neurologie II. Für später vormerken. 1912.
8. Tretiakoff C. Contribution a l'etude de l'anatomiepathologique du locus niger de Soemmering avec quelques deductions relatives a la pathologenie des troubles dutonus musculaire de lamaladiede Parkinson. Thèse, Paris. 1919.
9. Braak H, Del Tredici K. Neuropathological Staging of Brain Pathology in Sporadic Parkinson's disease: Separating the Wheat from the Chaff. J Parkinsons Dis. 2017;7(s1):S73-S87.
10. Shults CW. Lewy bodies. Proc Natl Acad Sci U S A. 2006;103(6):1661-8.
11. Vilchez D, Saez I, Dillin A. The role of protein clearance mechanisms in organismal ageing and age-related diseases. Nat Commun. 2014;5:5659.
12. Mercado G, Castillo V, Soto P, Sidhu A. ER stress and Parkinson's disease: Pathological inputs that converge into the secretory pathway. Brain Res. 2016;1648(Pt B):626-32.
13. Cai Y, Arikkath J, Yang L, Guo ML, Periyasamy P, Buch S. Interplay of endoplasmic reticulum stress and autophagy in neurodegenerative disorders. Autophagy. 2016;12(2):225-44.
14. Truban D, Hou X, Caulfield TR, Fiesel FC, Springer W. PINK1, Parkin, and Mitochondrial Quality Control: What can we Learn about Parkinson's Disease Pathobiology? J Parkinsons Dis. 2017;7(1):13-29.
15. Giannoccaro MP, La Morgia C, Rizzo G, Carelli V. Mitochondrial DNA and primary mitochondrial dysfunction in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2017;32(3):346-63.
16. Bose A, Beal MF. Mitochondrial dysfunction in Parkinson's disease. J Neurochem. 2016;139 Suppl 1:216-31.
17. Tiwari PC, Pal R. The potential role of neuroinflammation and transcription factors in Parkinson disease. Dialogues in clinical neuroscience. 2017;19(1):71-80.
18. Joshi N, Singh S. Updates on immunity and inflammation in Parkinson disease pathology. Journal of neuroscience research. 2017.

19. Duda J, Potschke C, Liss B. Converging roles of ion channels, calcium, metabolic stress, and activity pattern of Substantia nigra dopaminergic neurons in health and Parkinson's disease. J Neurochem. 2016;139 Suppl 1:156-78.

20. Pfeiffer RF. Non-motor symptoms in Parkinson's disease. Parkinsonism Relat Disord. 2016;22 Suppl 1:S119-22.

21. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rüb U. Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). Journal of Neurology. 2002;249(0):1-.

22. Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res. 2004;318(1):121-34.

23. Markesbery WR, Jicha GA, Liu H, Schmitt FA. Lewy body pathology in normal elderly subjects. J Neuropathol Exp Neurol. 2009;68(7):816-22.

24. Sasaki S, Shirata A, Yamane K, Iwata M. Parkin-positive autosomal recessive juvenile Parkinsonism with alpha-synuclein-positive inclusions. Neurology. 2004;63(4):678-82.

25. Hayashi S, Wakabayashi K, Ishikawa A, Nagai H, Saito M, Maruyama M, et al. An autopsy case of autosomal-recessive juvenile parkinsonism with a homozygous exon 4 deletion in the parkin gene. Movement disorders : official journal of the Movement Disorder Society. 2000;15(5):884-8.

26. Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with parkinsonism: Review of the literature. Movement disorders : official journal of the Movement Disorder Society. 2017;32(11):1504-23.

27. Surmeier DJ, Obeso JA, Halliday GM. Selective neuronal vulnerability in Parkinson disease. Nat Rev Neurosci. 2017;18(2):101-13.

28. Buchman AS, Shulman JM, Nag S, Leurgans SE, Arnold SE, Morris MC, et al. Nigral pathology and parkinsonian signs in elders without Parkinson disease. Ann Neurol. 2012;71(2):258-66.

29. Cabello CR, Thune JJ, Pakkenberg H, Pakkenberg B. Ageing of substantia nigra in humans: cell loss may be compensated by hypertrophy. Neuropathol Appl Neurobiol. 2002;28(4):283-91.

30. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain. 1991;114 (Pt 5):2283-301.

31. Fedorow H, Tribl F, Halliday G, Gerlach M, Riederer P, Double KL. Neuromelanin in human dopamine neurons: comparison with peripheral melanins and relevance to Parkinson's disease. Prog Neurobiol. 2005;75(2):109-24.

32. Ma SY, Roytt M, Collan Y, Rinne JO. Unbiased morphometrical measurements show loss of pigmented nigral neurones with ageing. Neuropathol Appl Neurobiol. 1999;25(5):394-9.

33. Naoi M, Maruyama W. Cell death of dopamine neurons in aging and Parkinson's disease. Mech Ageing Dev. 1999;111(2-3):175-88.

34. Rudow G, O'Brien R, Savonenko AV, Resnick SM, Zonderman AB, Pletnikova O, et al. Morphometry of the human substantia nigra in ageing and Parkinson's disease. Acta neuropathologica. 2008;115(4):461-70.

35. Stark AK, Pakkenberg B. Histological changes of the dopaminergic nigrostriatal system in aging. Cell Tissue Res. 2004;318(1):81-92.

36. Thiessen B, Rajput AH, Laverty W, Desai H. Age, environments, and the number of substantia nigra neurons. Adv Neurol. 1990;53:201-6.

37. Ransmayr G, Faucheux B, Nowakowski C, Kubis N, Federspiel S, Kaufmann W, et al. Age-related changes of neuronal counts in the human pedunculopontine nucleus. Neuroscience Letters. 2000;288(3):195-8.

38. Lohr JB, Jeste DV. Locus ceruleus morphometry in aging and schizophrenia. Acta Psychiatr Scand. 1988;77(6):689-97.

39. Shibata E, Sasaki M, Tohyama K, Kanbara Y, Otsuka K, Ehara S, et al. Age-related changes in locus ceruleus on neuromelanin magnetic resonance imaging at 3 Tesla. Magn Reson Med Sci. 2006;5(4):197-200.

40. Goldstein DS. Cardiac denervation in patients with Parkinson disease. Cleveland Clinic journal of medicine. 2007;74 Suppl 1:S91-4.

41. Wong KK, Raffel DM, Koeppe RA, Frey KA, Bohnen NI, Gilman S. Pattern of cardiac sympathetic denervation in idiopathic Parkinson disease studied with 11C hydroxyephedrine PET. Radiology. 2012;265(1):240-7.

42. Orimo S, Oka T, Miura H, Tsuchiya K, Mori F, Wakabayashi K, et al. Sympathetic cardiac denervation in Parkinson's disease and pure autonomic failure but not in multiple system atrophy. J Neurol Neurosurg Psychiatry. 2002;73(6):776-7.

43. Dijkstra AA, Voorn P, Berendse HW, Groenewegen HJ, Netherlands Brain B, Rozemuller AJ, et al. Stage-dependent nigral neuronal loss in incidental Lewy body and Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(10):1244-51.

44. Iacono D, Geraci-Erck M, Rabin ML, Adler CH, Serrano G, Beach TG, et al. Parkinson disease and incidental Lewy body disease: Just a question of time? Neurology. 2015;85(19):1670-9.

45. Milber JM, Noorigian JV, Morley JF, Petrovitch H, White L, Ross GW, et al. Lewy pathology is not the first sign of degeneration in vulnerable neurons in Parkinson disease. Neurology. 2012;79(24):2307-14.

46. Gibb WR, Fearnley JM, Lees AJ. The anatomy and pigmentation of the human substantia nigra in relation to selective neuronal vulnerability. Adv Neurol. 1990;53:31-4.

47. Rinne JO, Ma SY, Lee MS, Collan Y, Roytta M. Loss of cholinergic neurons in the pedunculopontine nucleus in Parkinson's disease is related to disability of the patients. Parkinsonism & Related Disorders. 2008;14(7):553-7.

48. Karachi C, Grabli D, Bernard FA, Tande D, Wattiez N, Belaid H, et al. Cholinergic mesencephalic neurons are involved in gait and postural disorders in Parkinson disease. J Clin Invest. 2010;120(8):2745-54.

49. Hepp DH, Ruiter AM, Galis Y, Voorn P, Rozemuller AJM, Berendse HW, et al. Pedunculopontine Cholinergic Cell Loss in Hallucinating Parkinson Disease Patients but Not in Dementia With Lewy Bodies Patients. Journal of Neuropathology and Experimental Neurology. 2013;72(12):1162-70.

50. Pienaar IS, Elson JL, Racca C, Nelson G, Turnbull DM, Morris CM. Mitochondrial abnormality associates with type-specific neuronal loss and cell morphology changes in the pedunculopontine nucleus in Parkinson disease. Am J Pathol. 2013;183(6):1826-40.

51. Gai WP, Halliday GM, Blumbergs PC, Geffen LB, Blessing WW. Substance P-containing neurons in the mesopontine tegmentum are severely affected in Parkinson's disease. Brain. 1991;114 (Pt 5):2253-67.

52. Bertrand E, Lechowicz W, Szpak GM, Dymecki J. Qualitative and quantitative analysis of locus coeruleus neurons in Parkinson's disease. Folia neuropathologica. 1997;35(2):80-6.

53. Gai WP, Blumbergs PC, Geffen LB, Blessing WW. Age-related loss of dorsal vagal neurons in Parkinson's disease. Neurology. 1992;42(11):2106-11.

54. Cheshire P, Ayton S, Bertram KL, Ling H, Li A, McLean C, et al. Serotonergic markers in Parkinson's disease and levodopa-induced dyskinesias. Movement disorders : official journal of the Movement Disorder Society. 2015;30(6):796-804.

55. McRitchie DA, Cartwright HR, Halliday GM. Specific A10 dopaminergic nuclei in the midbrain degenerate in Parkinson's disease. Exp Neurol. 1997;144(1):202-13.

56. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain. 1999;122 (Pt 8):1437-48.

57. Henderson JM, Carpenter K, Cartwright H, Halliday GM. Degeneration of the centre median-parafascicular complex in Parkinson's disease. Ann Neurol. 2000;47(3):345-52.

58. Halliday GM, Macdonald V, Henderson JM. A comparison of degeneration in motor thalamus and cortex between progressive supranuclear palsy and Parkinson's disease. Brain. 2005;128(Pt 10):2272-80.

59. Thannickal TC, Lai YY, Siegel JM. Hypocretin (orexin) cell loss in Parkinson's disease. Brain. 2007;130(Pt 6):1586-95.

60. Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 1995;10(3):283-7.

61. Huisman E, Uylings HB, Hoogland PV. A 100% increase of dopaminergic cells in the olfactory bulb may explain hyposmia in Parkinson's disease. Mov Disord. 2004;19(6):687-92.

62. Huisman E, Uylings HB, Hoogland PV. Gender-related changes in increase of dopaminergic neurons in the olfactory bulb of Parkinson's disease patients. Movement disorders : official journal of the Movement Disorder Society. 2008;23(10):1407-13.

63. Mundinano IC, Caballero MC, Ordonez C, Hernandez M, DiCaudo C, Marcilla I, et al. Increased dopaminergic cells and protein aggregates in the olfactory bulb of patients with neurodegenerative disorders. Acta neuropathologica. 2011;122(1):61-74.

64. Klingelhoefer L, Reichmann H. Pathogenesis of Parkinson disease--the gut-brain axis and environmental factors. Nat Rev Neurol. 2015;11(11):625-36.

65. Singaram C, Ashraf W, Gaumnitz EA, Torbey C, Sengupta A, Pfeiffer R, et al. Dopaminergic defect of enteric nervous system in Parkinson's disease patients with chronic constipation. Lancet. 1995;346(8979):861-4.

66. Annerino DM, Arshad S, Taylor GM, Adler CH, Beach TG, Greene JG. Parkinson's disease is not associated with gastrointestinal myenteric ganglion neuron loss. Acta neuropathologica. 2012;124(5):665-80.

67. Benarroch EE, Schmeichel AM, Sandroni P, Low PA, Parisi JE. Involvement of vagal autonomic nuclei in multiple system atrophy and Lewy body disease. Neurology. 2006;66(3):378-83.

68. Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol. 1997;38 Suppl 2:2-7.

69. Harding AJ, Stimson E, Henderson JM, Halliday GM. Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. Brain. 2002;125(Pt 11):2431-45.

70. Hirsch E, Graybiel AM, Agid YA. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature. 1988;334(6180):345-8.

71. German DC, Manaye K, Smith WK, Woodward DJ, Saper CB. Midbrain dopaminergic cell loss in Parkinson's disease: computer visualization. Ann Neurol. 1989;26(4):507-14.

72. Halliday GM, Li YW, Blumbergs PC, Joh TH, Cotton RG, Howe PR, et al. Neuropathology of immunohistochemically identified brainstem neurons in Parkinson's disease. Ann Neurol. 1990;27(4):373-85.

73. Paulus W, Jellinger K. The neuropathologic basis of different clinical subgroups of Parkinson's disease. J Neuropathol Exp Neurol. 1991;50(6):743-55.

74. Zarrow C, Lyness SA, Mortimer JA, Chui HC. Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases. Arch Neurol. 2003;60:337-41.

75. Halliday GM, Blumbergs PC, Cotton RG, Blessing WW, Geffen LB. Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. Brain Res. 1990;510(1):104-7.

76. Bolam JP, Pissadaki EK. Living on the edge with too many mouths to feed: Why dopamine neurons die. Movement disorders : official journal of the Movement Disorder Society. 2012;27(12):1478-83.

77. Surmeier DJ, Schumacker PT, Guzman JD, Ilijic E, Yang B, Zampese E. Calcium and Parkinson's disease. Biochem Biophys Res Commun. 2017;483(4):1013-9.

78. Pacelli C, Giguere N, Bourque MJ, Levesque M, Slack RS, Trudeau LE. Elevated Mitochondrial Bioenergetics and Axonal Arborization Size Are Key Contributors to the Vulnerability of Dopamine Neurons. Curr Biol. 2015;25(18):2349-60.

79. Segura-Aguilar J, Paris I, Munoz P, Ferrari E, Zecca L, Zucca FA. Protective and toxic roles of dopamine in Parkinson's disease. J Neurochem. 2014;129(6):898-915.

80. Van Laar VS, Mishizen AJ, Cascio M, Hastings TG. Proteomic identification of dopamine-conjugated proteins from isolated rat brain mitochondria and SH-SY5Y cells. Neurobiol Dis. 2009;34(3):487-500.

81. Xu Y, Stokes AH, Roskoski R, Jr., Vrana KE. Dopamine, in the presence of tyrosinase, covalently modifies and inactivates tyrosine hydroxylase. Journal of neuroscience research. 1998;54(5):691-7.

82. Whitehead RE, Ferrer JV, Javitch JA, Justice JB. Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. J Neurochem. 2001;76(4):1242-51.

83. Mosharov EV, Larsen KE, Kanter E, Phillips KA, Wilson K, Schmitz Y, et al. Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. Neuron. 2009;62(2):218-29.

84. Lohr KM, Bernstein AI, Stout KA, Dunn AR, Lazo CR, Alter SP, et al. Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo. Proc Natl Acad Sci U S A. 2014;111(27):9977-82.

85. Pifl C, Rajput A, Reither H, Blesa J, Cavada C, Obeso JA, et al. Is Parkinson's disease a vesicular dopamine storage disorder? Evidence from a study in isolated synaptic vesicles of human and nonhuman primate striatum. J Neurosci. 2014;34(24):8210-8.

86. Caudle WM, Richardson JR, Wang MZ, Taylor TN, Guillot TS, McCormack AL, et al. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J Neurosci. 2007;27(30):8138-48.

87. Guillot TS, Miller GW. Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. Mol Neurobiol. 2009;39(2):149-70.

88. Fahn S. Does levodopa slow or hasten the rate of progression of Parkinson's disease? J Neurol. 2005;252 Suppl 4:Iv37-iv42.

89. Lipski J, Nistico R, Berretta N, Guatteo E, Bernardi G, Mercuri NB. L-DOPA: a scapegoat for accelerated neurodegeneration in Parkinson's disease? Prog Neurobiol. 2011;94(4):389-407.

90. Haacke EM, Miao Y, Liu M, Habib CA, Katkuri Y, Liu T, et al. Correlation of putative iron content as represented by changes in R2* and phase with age in deep gray matter of healthy adults. J Magn Reson Imaging. 2010;32(3):561-76.

91. Daugherty A, Raz N. Age-related differences in iron content of subcortical nuclei observed in vivo: a meta-analysis. Neuroimage. 2013;70:113-21.

92. Bilgic B, Pfefferbaum A, Rohlfing T, Sullivan EV, Adalsteinsson E. MRI estimates of brain iron concentration in normal aging using quantitative susceptibility mapping. Neuroimage. 2012;59(3):2625-35.

93. Ren Y, Liu W, Jiang H, Jiang Q, Feng J. Selective vulnerability of dopaminergic neurons to microtubule depolymerization. J Biol Chem. 2005;280(40):34105-12.

94. Friedman A, Galazka-Friedman J, Bauminger ER. Iron as a trigger of neurodegeneration in Parkinson's disease. Handb Clin Neurol. 2007;83:493-505.

95. Sian-Hulsmann J, Mandel S, Youdim MB, Riederer P. The relevance of iron in the pathogenesis of Parkinson's disease. J Neurochem. 2011;118(6):939-57.

96. Kosta P, Argyropoulou MI, Markoula S, Konitsiotis S. MRI evaluation of the basal ganglia size and iron content in patients with Parkinson's disease. J Neurol. 2006;253(1):26-32.

97. Zecca L, Shima T, Stroppolo A, Goj C, Battiston GA, Gerbasi R, et al. Interaction of neuromelanin and iron in substantia nigra and other areas of human brain. Neuroscience. 1996;73(2):407-15.

98. Zucca FA, Bellei C, Giannelli S, Terreni MR, Gallorini M, Rizzio E, et al. Neuromelanin and iron in human locus coeruleus and substantia nigra during aging: consequences for neuronal vulnerability. J Neural Transm (Vienna). 2006;113(6):757-67.

99. Zecca L, Stroppolo A, Gatti A, Tampellini D, Toscani M, Gallorini M, et al. The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. Proc Natl Acad Sci U S A. 2004;101(26):9843-8.

100. Michel PP, Alvarez-Fischer D, Guerreiro S, Hild A, Hartmann A, Hirsch EC. Role of activity-dependent mechanisms in the control of dopaminergic neuron survival. J Neurochem. 2007;101(2):289-97.

101. Guzman JN, Sanchez-Padilla J, Wokosin D, Kondapalli J, Ilijic E, Schumacker PT, et al. Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature. 2010;468(7324):696-700.

102. Goldberg JA, Guzman JN, Estep CM, Ilijic E, Kondapalli J, Sanchez-Padilla J, et al. Calcium entry induces mitochondrial oxidant stress in vagal neurons at risk in Parkinson's disease. Nat Neurosci. 2012;15(10):1414-21.

103. Sanchez-Padilla J, Guzman JN, Ilijic E, Kondapalli J, Galtieri DJ, Yang B, et al. Mitochondrial oxidant stress in locus coeruleus is regulated by activity and nitric oxide synthase. Nat Neurosci. 2014;17(6):832-40.

104. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: Why is advancing age the biggest risk factor?☆. Ageing Res Rev. 142014. p. 19-30.

105. Hell JW, Westenbroek RE, Warner C, Ahlijanian MK, Prystay W, Gilbert MM, et al. Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel alpha 1 subunits. J Cell Biol. 1993;123(4):949-62.

106. Hurley MJ, Gentleman SM, Dexter DT. Calcium CaV1 channel subtype mRNA expression in Parkinson's disease examined by in situ hybridization. Journal of molecular neuroscience : MN. 2015;55(3):715-24.

107. Schiemann J, Schlaudraff F, Klose V, Bingmer M, Seino S, Magill PJ, et al. K-ATP channels in dopamine substantia nigra neurons control bursting and novelty-induced exploration. Nat Neurosci. 2012;15(9):1272-80.

108. Liss B, Haeckel O, Wildmann J, Miki T, Seino S, Roeper J. K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. Nat Neurosci. 2005;8(12):1742-51.

109. Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, et al. Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. J Neurosci. 2009;29(2):444-53.

110. Pissadaki EK, Bolam JP. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. Front Comput Neurosci. 2013;7:13.

111. Takakusaki K, Shiroyama T, Yamamoto T, Kitai ST. Cholinergic and noncholinergic tegmental pedunculopontine projection neurons in rats revealed by intracellular labeling. The Journal of comparative neurology. 1996;371(3):345-61.

112. Gao K, Mason P. Somatodendritic and axonal anatomy of intracellularly labeled serotonergic neurons in the rat medulla. The Journal of comparative neurology. 1997;389(2):309-28.

113. Gao H, Glatzer NR, Williams KW, Derbenev AV, Liu D, Smith BN. Morphological and electrophysiological features of motor neurons and putative interneurons in the dorsal vagal complex of rats and mice. Brain Res. 2009;1291:40-52.

114. Gagnon D, Parent M. Distribution of VGLUT3 in highly collateralized axons from the rat dorsal raphe nucleus as revealed by single-neuron reconstructions. PLoS One. 2014;9(2):e87709.

115. Chazal G, Ma W. An ultrastructural analysis of serotoninergic neurons in the nucleus raphe magnus of the rat. Neuroscience. 1989;33(2):301-10.

116. Jones BE, Yang TZ. The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. The Journal of comparative neurology. 1985;242(1):56-92.

117. Contant C, Umbriaco D, Garcia S, Watkins KC, Descarries L. Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. Neuroscience. 1996;71(4):937-47.

118. Zhou FM, Wilson CJ, Dani JA. Cholinergic interneuron characteristics and nicotinic properties in the striatum. Journal of neurobiology. 2002;53(4):590-605.

119. Meitzen J, Pflepsen KR, Stern CM, Meisel RL, Mermelstein PG. Measurements of neuron soma size and density in rat dorsal striatum, nucleus accumbens core and nucleus accumbens shell: differences between striatal region and brain hemisphere, but not sex. Neurosci Lett. 2011;487(2):177-81.

120. Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol. 1994;36(3):348-55.

121. Pearce RK, Owen A, Daniel S, Jenner P, Marsden CD. Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. J Neural Transm (Vienna). 1997;104(6-7):661-77.

122. Sofic E, Lange KW, Jellinger K, Riederer P. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci Lett. 1992;142(2):128-30.

123. Tagliaferro P, Burke RE. Retrograde Axonal Degeneration in Parkinson Disease. J Parkinsons Dis. 2016;6(1):1-15.

124. O'Malley KL. The role of axonopathy in Parkinson's disease. Exp Neurobiol. 2010;19(3):115-9.

125. Kinghorn KJ, Asghari AM, Castillo-Quan JI. The emerging role of autophagic-lysosomal dysfunction in Gaucher disease and Parkinson's disease. Neural regeneration research. 2017;12(3):380-4.

126. Wong YC, Krainc D. Lysosomal trafficking defects link Parkinson's disease with Gaucher's disease. Movement disorders : official journal of the Movement Disorder Society. 2016;31(11):1610-8.

127. Migdalska-Richards A, Schapira AH. The relationship between glucocerebrosidase mutations and Parkinson disease. J Neurochem. 2016;139 Suppl 1:77-90.

128. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. The New England journal of medicine. 2009;361(17):1651-61.

129. Gegg ME, Burke D, Heales SJ, Cooper JM, Hardy J, Wood NW, et al. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. Ann Neurol. 2012;72(3):455-63.

130. Murphy KE, Halliday GM. Glucocerebrosidase deficits in sporadic Parkinson disease. Autophagy. 2014;10(7):1350-1.

131. Dehay B, Martinez-Vicente M, Caldwell GA, Caldwell KA, Yue Z, Cookson MR, et al. Lysosomal impairment in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2013;28(6):725-32.

132. Ashrafi G, Schwarz TL. PINK1- and PARK2-mediated local mitophagy in distal neuronal axons. Autophagy. 2015;11(1):187-9.

133. Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, et al. Parkinson's Disease-Related Proteins PINK1 and Parkin Repress Mitochondrial Antigen Presentation. Cell. 2016;166(2):314-27.

134. Sheng C, Heng X, Zhang G, Xiong R, Li H, Zhang S, et al. DJ-1 deficiency perturbs microtubule dynamics and impairs striatal neurite outgrowth. Neurobiology of aging. 2013;34(2):489-98.

135. Di Nottia M, Masciullo M, Verrigni D, Petrillo S, Modoni A, Rizzo V, et al. DJ-1 modulates mitochondrial response to oxidative stress: clues from a novel diagnosis of PARK7. Clin Genet. 2017;92(1):18-25.

136. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. Neuron. 2006;52(4):587-93.

137. Yue M, Hinkle KM, Davies P, Trushina E, Fiesel FC, Christenson TA, et al. Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock-in mice. Neurobiol Dis. 2015;78:172-95.

138. Surmeier DJ, Halliday GM, Simuni T. Calcium, mitochondrial dysfunction and slowing the progression of Parkinson's disease. Exp Neurol. 2017;298(Pt B):202-9.

139. Wang Z, Hou L, Huang L, Guo J, Zhou X. Exenatide improves liver mitochondrial dysfunction and insulin resistance by reducing oxidative stress in high fat diet-induced obese mice. Biochem Biophys Res Commun. 2017;486(1):116-23.

140. Li Y, Perry T, Kindy MS, Harvey BK, Tweedie D, Holloway HW, et al. GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. Proc Natl Acad Sci U S A. 2009;106(4):1285-90.

141. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. Lancet. 2017;390(10103):1664-75.

142. Gleave JA, Arathoon LR, Trinh D, Lizal KE, Giguere N, Barber JHM, et al. Sirtuin 3 rescues neurons through the stabilisation of mitochondrial biogenetics in the virally-expressing mutant alpha-synuclein rat model of parkinsonism. Neurobiol Dis. 2017;106:133-46.

143. Shi H, Deng HX, Gius D, Schumacker PT, Surmeier DJ, Ma YC. Sirt3 protects dopaminergic neurons from mitochondrial oxidative stress. Hum Mol Genet. 2017;26(10):1915-26.

144. Greenfield JG, Bosanquet FD. The brain-stem lesions in Parkinsonism. J Neurol Neurosurg Psychiatry. 1953;16(4):213-26.

145. Pakkenberg H, Brody H. The number of nerve cells in the substantia nigra in paralysis agitans. Acta neuropathologica. 1965;5(2):320-4.

146. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci. 1973;20(4):415-55.

147. Rajput AH, Rozdilsky B. Dysautonomia in Parkinsonism: a clinicopathological study. J Neurol Neurosurg Psychiatry. 1976;39(11):1092-100.

148. Gaspar P, Gray F. Dementia in idiopathic Parkinson's disease. A neuropathological study of 32 cases. Acta neuropathologica. 1984;64(1):43-52.

149. Tagliavini F, Pilleri G, Bouras C, Constantinidis J. The basal nucleus of Meynert in idiopathic Parkinson's disease. Acta neurologica Scandinavica. 1984;70(1):20-8.

150. Chan-Palay V. Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia. The Journal of comparative neurology. 1988;273(4):543-57.

151. Gibb WR, Lees AJ. A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. Neurology. 1988;38(9):1402-6.

152. Rinne JO, Rummukainen J, Paljarvi L, Rinne UK. Dementia in Parkinson's disease is related to neuronal loss in the medial substantia nigra. Ann Neurol. 1989;26(1):47-50.

153. Zweig RM, Jankel WR, Hedreen JC, Mayeux R, Price DL. The pedunculopontine nucleus in Parkinson's disease. Ann Neurol. 1989;26(1):41-6.

154. Pakkenberg B, Moller A, Gundersen HJ, Mouritzen Dam A, Pakkenberg H. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. J Neurol Neurosurg Psychiatry. 1991;54(1):30-3.

155. Xuereb JH, Perry RH, Candy JM, Perry EK, Marshall E, Bonham JR. Nerve cell loss in the thalamus in Alzheimer's disease and Parkinson's disease. Brain. 1991;114 (Pt 3):1363-79.

156. Moller A. Mean volume of pigmented neurons in the substantia nigra in Parkinson's disease. Acta Neurol Scand Suppl. 1992;137:37-9.

157. Zweig RM, Cardillo JE, Cohen M, Giere S, Hedreen JC. The locus ceruleus and dementia in Parkinson's disease. Neurology. 1993;43(5):986-91.

158. Mouatt-Prigent A, Agid Y, Hirsch EC. Does the calcium binding protein calretinin protect dopaminergic neurons against degeneration in Parkinson's disease? Brain Res. 1994;668(1-2):62-70.

159. Ma SY, Collan Y, Roytta M, Rinne JO, Rinne UK. Cell counts in the substantia nigra: a comparison of single section counts and disector counts in patients with Parkinson's disease and in controls. Neuropathol Appl Neurobiol. 1995;21(1):10-7.

160. Halliday GM, McRitchie DA, Cartwright H, Pamphlett R, Hely MA, Morris JG. Midbrain neuropathology in idiopathic Parkinson's disease and diffuse Lewy body disease. J Clin Neurosci. 1996;3(1):52-60.

161. Ma SY, Rinne JO, Collan Y, Roytta M, Rinne UK. A quantitative morphometrical study of neuron degeneration in the substantia nigra in Parkinson's disease. J Neurol Sci. 1996;140(1-2):40-5.

162. Ma SY, Roytta M, Rinne JO, Collan Y, Rinne UK. Correlation between neuromorphometry in the substantia nigra and clinical features in Parkinson's disease using disector counts. J Neurol Sci. 1997;151(1):83-7.

163. Greffard S, Verny M, Bonnet AM, Beinis JY, Gallinari C, Meaume S, et al. Motor score of the Unified Parkinson Disease Rating Scale as a good predictor of Lewy body-associated neuronal loss in the substantia nigra. Arch Neurol. 2006;63(4):584-8.

164. Beach TG, Adler CH, Lue L, Sue LI, Bachalakuri J, Henry-Watson J, et al. Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta neuropathologica. 2009;117(6):613-34.

165. Kordower JH, Olanow CW, Dodiya HB, Chu Y, Beach TG, Adler CH, et al. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. Brain. 2013;136(Pt 8):2419-31.

166. Kraemmer J, Kovacs GG, Perju-Dumbrava L, Pirker S, Traub-Weidinger T, Pirker W. Correlation of striatal dopamine transporter imaging with post mortem substantia nigra cell counts. Movement disorders : official journal of the Movement Disorder Society. 2014;29(14):1767-73.

167. Chan-Palay V, Asan E. Alterations in catecholamine neurons of the locus coeruleus in senile dementia of the Alzheimer type and in Parkinson's disease with and without dementia and depression. The Journal of comparative neurology. 1989;287(3):373-92.

168. German DC, Manaye KF, White CL, 3rd, Woodward DJ, McIntire DD, Smith WK, et al. Disease-specific patterns of locus coeruleus cell loss. Ann Neurol. 1992;32(5):667-76.

169. Patt S, Gerhard L. A Golgi study of human locus coeruleus in normal brains and in Parkinson's disease. Neuropathol Appl Neurobiol. 1993;19(6):519-23.

170. Hoogendijk WJ, Pool CW, Troost D, van Zwieten E, Swaab DF. Image analyser-assisted morphometry of the locus coeruleus in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Brain. 1995;118 (Pt 1):131-43.

171. Brunnstrom H, Friberg N, Lindberg E, Englund E. Differential degeneration of the locus coeruleus in dementia subtypes. Clinical neuropathology. 2011;30(3):104-10.

172. McMillan PJ, White SS, Franklin A, Greenup JL, Leverenz JB, Raskind MA, et al. Differential response of the central noradrenergic nervous system to the loss of locus coeruleus neurons in Parkinson's disease and Alzheimer's disease. Brain Res. 2011;1373:240-52.

173. Dugger BN, Murray ME, Boeve BF, Parisi JE, Benarroch EE, Ferman TJ, et al. Neuropathological analysis of brainstem cholinergic and catecholaminergic nuclei in relation to rapid eye movement (REM) sleep behaviour disorder. Neuropathol Appl Neurobiol. 2012;38(2):142-52.

174. Del Tredici K, Braak H. Dysfunction of the locus coeruleus-norepinephrine system and related circuitry in Parkinson's disease-related dementia. J Neurol Neurosurg Psychiatry. 2013;84(7):774-83.

175. Arendt T, Bigl V, Arendt A, Tennstedt A. Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's Disease. Acta neuropathologica. 1983;61(2):101-8.

176. Candy JM, Perry RH, Perry EK, Irving D, Blessed G, Fairbairn AF, et al. Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases. J Neurol Sci. 1983;59(2):277-89.

177. Nakano I, Hirano A. Neuron loss in the nucleus basalis of Meynert in parkinsonism-dementia complex of Guam. Ann Neurol. 1983;13(1):87-91.

178. Whitehouse PJ, Hedreen JC, White CL, 3rd, Price DL. Basal forebrain neurons in the dementia of Parkinson disease. Ann Neurol. 1983;13(3):243-8.

179. Nakano I, Hirano A. Parkinson's disease: Neuron loss in the nucleus basalis withouth concomitant Alzheimer’s Disease. Ann Neurol. 1984;15(5):415-8.

180. Perry EK, Curtis M, Dick DJ, Candy JM, Atack JR, Bloxham CA, et al. Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease. J Neurol Neurosurg Psychiatry. 1985;48(5):413-21.

181. Rogers JD, Brogan D, Mirra SS. The nucleus basalis of Meynert in neurological disease: a quantitative morphological study. Ann Neurol. 1985;17(2):163-70.

182. Hirsch EC, Graybiel AM, Duyckaerts C, Javoy-Agid F. Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. Proc Natl Acad Sci U S A. 1987;84(16):5976-80.

183. Jellinger K. The pedunculopontine nucleus in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease. J Neurol Neurosurg Psychiatry. 1988;51(4):540-3.

184. Schmeichel AM, Buchhalter LC, Low PA, Parisi JE, Boeve BW, Sandroni P, et al. Mesopontine cholinergic neuron involvement in Lewy body dementia and multiple system atrophy. Neurology. 2008;70(5):368-73.

185. Pienaar IS, Elson JL, Racca C, Nelson G, Turnbull DM, Morris CM. Mitochondrial abnormality associates with type-specific neuronal loss and cell morphology changes in the pedunculopontine nucleus in Parkinson disease. Am J Pathol. 2013;183(6):1826-40.

186. Kremer HP. The hypothalamic lateral tuberal nucleus: normal anatomy and changes in neurological diseases. Prog Brain Res. 1992;93:249-61.

187. Kremer HP, Bots GT. Lewy bodies in the lateral hypothalamus: do they imply neuronal loss? Movement disorders : official journal of the Movement Disorder Society. 1993;8(3):315-20.

188. Purba JS, Hofman MA, Swaab DF. Decreased number of oxytocin-immunoreactive neurons in the paraventricular nucleus of the hypothalamus in Parkinson's disease. Neurology. 1994;44(1):84-9.

189. Nakamura S, Ohnishi K, Nishimura M, Suenaga T, Akiguchi I, Kimura J, et al. Large neurons in the tuberomammillary nucleus in patients with Parkinson's disease and multiple system atrophy. Neurology. 1996;46(6):1693-6.

190. Ansorge O, Daniel SE, Pearce RK. Neuronal loss and plasticity in the supraoptic nucleus in Parkinson's disease. Neurology. 1997;49(2):610-3.

191. Hoogendijk WJ, Purba JS, Hofman MA, de Vos RA, Jansen EN, Swaab DF. Depression in Parkinson's disease is not accompanied by more corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus. Biol Psychiatry. 1998;43(12):913-7.

192. Fronczek R, Overeem S, Lee SY, Hegeman IM, van Pelt J, van Duinen SG, et al. Hypocretin (orexin) loss in Parkinson's disease. Brain. 2007;130(Pt 6):1577-85.

193. Eadie MJ. The Pathology of Certain Medullary Nuclei in Parkinsonism. Brain. 1963;86:781-92.

194. Saper CB, Sorrentino DM, German DC, de Lacalle S. Medullary catecholaminergic neurons in the normal human brain and in Parkinson's disease. Ann Neurol. 1991;29(6):577-84.

195. Yamamoto T, Hirano A. Nucleus raphe dorsalis in parkinsonism-dementia complex of Guam. Acta neuropathologica. 1985;67(3-4):296-9.

196. Benarroch EE, Schmeichel AM, Sandroni P, Parisi JE, Low PA. Rostral raphe involvement in Lewy body dementia and multiple system atrophy. Acta neuropathologica. 2007;114(3):213-20.

197. Javoy-Agid F, Ruberg M, Taquet H, Bokobza B, Agid Y, Gaspar P, et al. Biochemical neuropathology of Parkinson's disease. Adv Neurol. 1984;40:189-98.

198. Dymecki J, Lechowicz W, Bertrand E, Szpak GM. Changes in dopaminergic neurons of the mesocorticolimbic system in Parkinson's disease. Folia neuropathologica. 1996;34(2):102-6.

199. Henderson JM, Carpenter K, Cartwright H, Halliday GM. Loss of thalamic intralaminar nuclei in progressive supranuclear palsy and Parkinson's disease: clinical and therapeutic implications. Brain. 2000;123 (Pt 7):1410-21.

200. Pedersen KM, Marner L, Pakkenberg H, Pakkenberg B. No global loss of neocortical neurons in Parkinson's disease: a quantitative stereological study. Mov Disord. 2005;20(2):164-71.

201. MacDonald V, Halliday GM. Selective loss of pyramidal neurons in the pre-supplementary motor cortex in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2002;17(6):1166-73.

202. Joelving FC, Billeskov R, Christensen JR, West M, Pakkenberg B. Hippocampal neuron and glial cell numbers in Parkinson's disease--a stereological study. Hippocampus. 2006;16(10):826-33.

12 Chapter 3 — Letter to the editor

Overview

Given the importance of the identity of the neurons degenerating in PD, we noticed that citations to our review on the evidence for cell loss in PD were often inaccurate or missing the point. Because we believe this to be such an essential element for understanding PD, we thought it pertinent to demonstrate to our research community that this crucial observation is not being addressed in the current literature. For the most part, claims made citing our work were for background information on PD. However, it became apparent that some of the claims made, citing our work, implied the contrary of what we had aimed to highlight. With hindsight, we could have made our observations more assertive, and clear; including a review on selective vulnerability in the review perhaps diluted the core message we had hoped to convey. In all, this letter hopes to illustrate what is perhaps a dogmatic view of selective vulnerability in PD, and to contribute to our community recognizing the need for further investigation into the identity of "PD-vulnerable neurons".

Contributions

Samuel Burke: conception, data collection, analysis, writing of manuscript.

Louis-Eric Trudeau: conception, providing resources, supervision, writing of manuscript.

Accepted subject to revisions (2021-11-17) at *npj Parkinson's Disease*

12.01 On cell loss in Parkinson's disease, and the citations that followed

Samuel Burke and Loui-Eric Trudeau

Writ large, Parkinson's disease (PD) is caused by the dysfunction and subsequent loss of several neuronal populations, notably dopamine neurons of the Substantia Nigra pars compacta (SNpc). Identifying the neurochemical and neuroanatomical identity of these neuronal populations — as well as the temporal order of their degeneration — is fundamental to understanding this disease.

It appears that many claims concerning the spatiotemporal pattern of neuronal loss in PD are based on Lewy pathology, rather than overt cell loss. However, counting neurons in post-mortem tissue is the only way to generate the data necessary required to understand properly such spatiotemporal pattern underlying the multiple symptoms of this disease. And the most (and only) reliable method for this is unbiased design-based stereology (1–3). Stereology as a method for 'neuromorphometry' has been around for well over a century (4), however, its application to the SNpc is comparatively recent (5), and much of the data on cell loss in PD precedes its widespread implementation. Not only has large parts of the literature on cell loss in PD not used design-based stereology, much has also not used simple standards of blinding and reporting essential clinical variables, let alone comparing cell counts of multiple neuronal populations in the same cases (6).

In 2018 we published a review article (6) where we aimed to communicate that, given the available literature, there is insufficient evidence to support granular claims made about the specific neurochemical identity of cells lost in the brains of people diagnosed with PD and to make conclusive and broad statements about the temporal order of this specific cell loss. Our goal was also to encourage the field to undertake further quantitative studies on neuronal loss in the human brain in PD, comparing multiple brain regions.

This review has gone on to be well cited (2021-09-24: Google Scholar: 136), yet, despite our presented data and arguments, statements citing this work very often do not integrate the view we have put forward. In fact, several even cite this piece in support of what is now most probably a dogma. Motivated by this observation, in this letter we aim to draw to the attention of basic and clinical scientists that the identity and temporal order of neuronal degeneration in PD is still an open question. Given that the specific identity and temporal order of degeneration underpins hypotheses of selective vulnerability — shaping the nature of comparative work trying to distinguish the features of neurons that render them vulnerable in PD and the paradigms that major funding bodies are funding — we believe it essential that new longitudinal studies be conducted addressing these questions.

Our aim here is to illustrate and understand the nature in which our previous review has impacted our field's understanding of PD. We note that most papers that cited our work, did so in support of

statements on general background information related to PD, and PD pathophysiology. However, we have noticed that very few claims integrate our core message: that as a field, we need to generate better quality data on the location and order of neuronal degeneration in PD.

Of the 136 citations listed by Google Scholar [at the time of writing] (the highest count across indexing platforms), we extracted 153 statements from 114 scientific documents citing our review (written in English and from peer-reviewed articles or theses). We then characterized these statements (Table 6) according to the criteria listed in Table 5.

Classification *Description*	Instances
Statements addressing broad background information on PD. *e.g. PD is associated with loss of dopamine (DA) neurons of the Substantia Nigra pars compacta.*	124
Statements addressing core message of review. *Referring to the lack of data on the spatiotemporal pattern of PD-specific cell loss.*	7
Statements made that are contrary to contents of review. *Inaccurate given the contents of our review. e.g. Ventral Tegmental Area dopamine neurons are unaffected in PD.*	7
Statements that are citing specific data from within the review. *Refers to specific data available from a study cited within our review.*	39
Statements made that are unrelated to review, or inaccurate. *Claiming specific loss, or lack of loss of neuronal types not mentioned in review.*	12

Table 5 Statements made citing our review categorized according to the described criteria.

Multiple statements made within a single publication were categorized individually.

The scarcity of statements made citing our core message suggests that our field is yet to integrate this message into its understanding of cell loss in PD. We observe that an equal number of statements — seven — are made addressing the core message of the review, as those that are inaccurate (continuing a narrative that this spatiotemporal pattern is well-established). Considering that how we cite can impact scientific narratives for decades (7,8), we hope that by addressing the nature of how the research community has taken up our initial message, we can contribute to accelerating progress in our field: avoiding epistemic cul-de-sacs driven by our research culture (9), and re-opening avenues towards progress.

We must highlight a notable exception by Huynh and colleagues (10) — whether impelled or not by our work — re-examined previous cases of a "well-characterized cohort". Acquiring bilateral brain stem samples (with detailed clinical records) from a previous study (11), Huynh et al. performed the first stereological quantifications of noradrenergic locus coeruleus neurons in confirmed cases of PD. Promisingly, for the sake of our models and in line with previous quantifications, they find substantial neuronal loss with ~43% of noradrenergic neurons remaining in this structure for cases with PD, and ~14% for cases of PD with dementia, compared to controls (with an *n* of 9, 5 and, 7 respectively). Notwithstanding, this study is unique, and it remains essential that we acquire more extensive longitudinal data on multiple neuronal populations, in the same cases, with detailed clinical variables measured.

At the time of writing, we have not received correspondence suggesting that we have missed vital literature, nor that our claims are unwarranted. Nevertheless, we have come to realize that literature was missed. With colleagues, a preregistered systematic review and meta-analysis (12) is ongoing to quantify cell loss in confirmed cases of PD across all studies available.

The following quote we believe embodies the urgency of addressing the points we raise: "What if our understanding of Parkinson's disease is also impeding our ability to find cures? Could it be that generating hypotheses based on what we think we know, along with our rigid funding models, is making it nearly impossible to find what we really need to know?" (13). As scientists, we must continue to serve the individuals at the end of our label — those diagnosed with PD, today and in the future.

12.02 Bibliography

1. Peterson, D. A. Stereology. in Encyclopedia of Movement Disorders (eds. Kompoliti, K. & Metman, L. V.) 168–170 (Academic Press, 2010). doi:10.1016/B978-0-12-374105-9.00287-2.
2. Schmitz, C. et al. Current automated 3D cell detection methods are not a suitable replacement for manual stereologic cell counting. Front. Neuroanat. 8, 27 (2014).
3. Jensen, E. B. V., Pakkenberg, B. & Baddeley, A. In memoriam – Hans Jørgen Gottlieb Gundersen. J. Microsc. 283, 169–177 (2021).
4. Haug, H. History of neuromorphometry. J. Neurosci. Methods 18, 1–17 (1986).
5. Ma, S. Y., Longo, F. M., Röyttä, M. & Collan, Y. Modern stereological evaluation in the aging human substantia nigra. Image Anal. Stereol. 22, 73–80 (2003).
6. Giguère, N., Burke Nanni, S. & Trudeau, L.-E. On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease. Front. Neurol. 9, 455 (2018).
7. Rekdal, O. B. Academic urban legends. Soc. Stud. Sci. 44, 638–654 (2014).
8. Rekdal, O. B. Monuments to Academic Carelessness: The Self-fulfilling Prophecy of Katherine Frost Bruner. Sci. Technol. Hum. Values 39, 744–758 (2014).
9. Nissen, S. B., Magidson, T., Gross, K. & Bergstrom, C. T. Publication bias and the canonization of false facts. eLife 5, e21451 (2016).
10. Huynh, B., Fu, Y., Kirik, D., Shine, J. M. & Halliday, G. M. Comparison of Locus Coeruleus Pathology with Nigral and Forebrain Pathology in Parkinson's Disease. Mov. Disord. (2021) doi:10.1002/mds.28615.
11. Hall, H. et al. Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. Brain J. Neurol. 137, 2493–2508 (2014).
12. Lunt, W. et al. Neurochemical identity and anatomy of cell loss in Parkinson's disease, and the clinical data available for correlation of progression : a systematic review and meta-analysis. PROSPERO 2021 (2021).
13. Espay, A. & Stecher, B. Brain Fables: The Hidden History of Neurodegenerative Diseases and a Blueprint to Conquer Them. (Cambridge University Press, 2020). doi:10.1017/9781108888202.

12.03 Statements citing our work

Statements	Publication	Classification
After the motor symptoms appear, nigral DA neuron loss increases up to 60% or higher and strongly correlates with the severity of motor features and disease duration. Apart from the SNpc, widespread cell loss can be found in several subcortical nuclei, including the locus coereleus, the nucleus basalis of Meynert, the dorsal motor nucleus of the vagus nerve, the pedunculopontine nucleus, the raphe nuclei, and also the hypothalamus and the olfactory bulb.	(Kouli et al., 2018)	background, background
The mechanisms underlying the selective and progressive mDAergic neuron death in PD and experimentally induced PD are not clarified; however, oxidative stress and inflammation associated to molecular changes indicative of mitochondrial dysfunction and apoptosis were identified in the parkinsonian brain. Earlier and more recent findings underscored that Wnt1/β-catenin signaling cascades play a prominent role in mDAergic development.	(Marchetti, 2018)	background, unrelated
PD is primarily characterized by a selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and their projections to the basal ganglia (BG) via the nigrostriatal pathway. Neurodegeneration has also been observed in other regions; including the locus coeruleus, basal forebrain, pedunculopontine nucleus, raphe nuclei, and hypothalamus.	(Assini, 2019)	background
The underlying cause of neurodegeneration in PD remains unknown. However, numerous studies have shown mitochondrial dysfunction to be associated with PD. Whether mitochondrial dysfunction is causative or simply a symptom of PD is still under investigation.	(Bastasic, 2019)	background, background, background
Its primary motor symptoms are caused by the progressive degeneration of dopaminergic midbrain neurons, particularly those within the substantia nigra (SN). This stressful Ca2+-driven mode of action distinguishes dopaminergic neurons in the SNc (and other vulnerable neurons) from neighboring pacemaking dopaminergic neurons in the ventral tegmental area (VTA), which are spared in Parkinson's disease.	(Benkert et al., 2019)	background, contrary
As a result, the degree of Parkinson's disease-related neuronal loss is likely just as severe in the LC as in the SNpc.	(Betts et al., 2019)	within, contrary [now is accurate with new data (2021) — but not at time of writing]
Though it is important to note that evidence connecting Lewy pathology and neuronal death is limited and neurodegeneration is an imperfect proxy for Lewy pathology.	(Blair et al., 2019)	background

Statements	Publication	Classification
… higher basal rate of mitochondrial oxidative phosphorylation … … axonal arborization, or afferent/efferent activity pattern …	(Blesa & Vila, 2019)	background, background
While other neuronal populations also undergo neurodegeneration in PD, the motor deficits resulting from nigrostriatal degeneration are the cardinal symptoms of PD and lead patients to seek medical attention. By the time that motor symptoms present, there is already significant loss of DAergic neurons in the nigrostriatal pathway.	(Bucher, 2019)	background, unrelated
While the accumulation of iron has not been proven as the sole cause of pathogenesis in these disorders, the fact that iron rich centers in the brain such as the basal ganglia are often more susceptible in neurodegenerative diseases suggests their potential involvement	(Deal & Yamamoto, 2019)	background
In this order of evidences, a recent review by Giguère et al. (2018) reveals that vulnerability of neuronal populations in PD extends well beyond basal ganglia and SNpc, these including the hippotalamus, locus coeruleous, pre-supplementary and premotor cortex, amygdala, thalamus, ventral tegmental area, raphe nuclei, dorsal motor nucleus of the vagus nerve, amongst others. Interestingly, no cell loss has been observed in the cerebellum.	(Díaz et al., 2019)	background, within
Some cells and regions within the brain are further vulnerable to inflammation for a variety of reasons including axonal length and complexity … The dopamine producing cells located in the SNc are one of these vulnerable populations. These areas notably include the SNc in PD as well as cholinergic neurons in LBD and are thought to be vulnerable to α-syn dysregulation and accumulation due to factors including: heightened metabolic rate. Parkinson's disease (PD) is characterized by a loss of substantia nigra pars compacta (SNc) dopaminergic neurons which project the basal ganglia, resulting in motor disturbances. Recent in vitro evidence suggests that the dopaminergic cells of the SNc may be particularly vulnerable to oxidative stress even compared to the relatively similar dopamine producing neurons of the ventral tegmental area, due to a variety of factors including activity, axonal length and mitochondrial density.	(Dwyer, 2019)	background, background, unrelated, background, within
At the time of PD diagnosis, it is estimated that between 50% and 70% of dopaminergic neurons within the SNpc have degenerated (Giguere et al., 2018), but by increasing the supply of exogenous LDOPA, the synthesis of dopamine can be increased in the surviving dopaminergic neurons.	(Giannopoulos et al., 2019)	background, within
PD is a neurodegenerative disorder primarily characterized by a massive loss of DA neurons in the SNc that is also thought to be accompanied by the loss of other types of neurons in a select subset of brain regions including the locus coeruleus and the pedunculopontine nucleus. A striking example of this selectivity is the much higher resilience of the neighboring DA neurons of the ventral tegmental area (VTA), which are far less affected than SNc DA neurons in PD.	(Giguère et al., 2019)	self-citation, background, within

Statements	Publication	Classification
Aside from the SNc, PD-specific cell loss has also been reported in the ventral tegmental area, amygdala and dorsal motor nucleus of the vagus nerve, hypothalamus, cortex, thalamus and many other brain regions.	(Goh et al., 2019)	background
In PD the predominant neurodegeneration involves dopamine neurons in the SNpc ventro-lateral tier.	(Hernandez et al., 2019)	background, within, within
There has also been some controversy about the 'neuroprotective' role of calbindin, since some studies have reported its presence in surviving neurons, but the expression pattern does not accurately reflect the different degrees of dopamine neuron vulnerability.		
Additionally, in PD animal models has been shown that complex axonal arborization, elevated mitochondrial bioenergetics (other references), and selective vulnerability of neuronal populations could contribute to the speed of progression of neurodegeneration.	(Hernandez-Baltazar et al., 2019)	background
These neurons contain calbindin (CAB) and glycolytic enzymes, but are poor in DAT and arborize profusely in the striatum and extrastriatal components of the BG. NM lipid changes, upregulation of α-Syn, low intrinsic calcium buffering capacity, change in iron levels, long, poorly myelinated, highly branched axons, and various risk factors promote the susceptibility to selective death of these neurons due to disruption of nuclear membrane integrity.	(Jellinger, 2019)	Background, within
However, relative vulnerability of some types of cells over others to the disease causing mechanisms account for the specific loss of dopamine neurons in the substantia nigra.	(Korecka et al., 2019)	background, unrelated
After the motor features appear, nigral DA neuron loss increases up to 60% or higher and strongly correlates with the severity of motor features and disease duration.	(Kouli, 2019)	within, contrary, background, within, background
Apart from the SNpc, widespread cell loss can be found in several other brain areas, including the locus coereleus, the nucleus basalis of Meynert, the dorsal motor nucleus of the vagus nerve, the pedunculopontine nucleus, the raphe nuclei, and also the hypothalamus and the olfactory bulb.		
Investigating axons with large arbours is important for understanding Parkinson's disease (PD).	(Kuznetsov & Kuznetsov, 2019)	Background, within
On the other hand, nondopamine neuronal populations are also highly affected by the degenerative process in PD.	(Liss & Striessnig, 2019)	background
Additionally, degeneration of locus coeruleus (LC) neurons, which utilize NE as neurotransmitter, occurs in PD and may precede that of SNpc DAneurons.	(Monzani et al., 2019)	background, contrary
Since the metabolic demands of SNc neurons are particularly high when compared to any other neuronal types (Sulzer, 2007) including neurons of other dopaminergic systems (Bolam and Pissadaki, 2012; Pacelli et al., 2015; Giguère et al., 2018), any sustained insufficiency in the supply of energy can result in cellular degeneration, characteristic of PD.	(Muddapu et al., 2019)	background, unrelated

Statements	Publication	Classification
Therefore, the insight behind these new lines of investigation is the mismatch in energy supply and demand which could be a primary factor underlying neurodegeneration in PD. Such a mismatch is more likely to take place in special nuclei like SNc due to their peculiar metabolic vulnerability.		
Parkinson's disease (PD) is characterized by progressive loss of the nigral dopaminergic neurons and pathological intraneuronal accumulation of α-synuclein (α-syn) in Lewy bodies in different areas of the nervous system.	(Nissen et al., 2019)	background
Although unbiased stereological quantifications are lacking, reported LC cell loss ranges from 21% to 93% and is commonly observed in advanced PD stages.	(Oertel et al., 2019)	core, within
PD typically starts with neuromelanin-rich dopaminergic neurons (DANs) degeneration in the substantia nigra pars compacta of the midbrain and dopamine (DA) deficiency in the striatum …	(Ren et al., 2019)	background, contrary, within
Loss of these cells causes the characteristic motor symptoms of the disease. In contrast, non-DA neuronal populations, such as cortical (CTX) neurons, are generally spared in the disease.	(Riessland et al., 2019)	Background, within
Its motor-related symptoms are caused by a progressive loss of dopaminergic (DA) neurons, particularly within the Substantia nigra (SN).	(Simons et al., 2019, p. 1)	background
Nigral dopaminergic neurons, and other specific cell types, are highly vulnerable in Parkinson's disease.	(Snyder, 2019)	background
Extensively cited.	(Wong et al., 2019)	Self-citation
Among several hypotheses regarding the selective neuronal vulnerability in PD, the autonomous pacemaking is gaining attention.	(Zhan et al., 2019)	background, within
The most prominent aspect is the loss of dopaminergic neurons in the substantia nigra pars compacta.	(Alegre-Cortés et al., 2020)	background
Apart from the SNpc, widespread cell loss can be found in several subcortical nuclei, including the locus coeruleus, the nucleus basalis of Meynert, the dorsal motor nucleus of the vagus nerve, the pedunculopontine nucleus, the raphe nuclei, the hypothalamus, and the olfactory bulp.	(Aryal et al., 2020)	background
PD is a multisystemic disorder since Lewy pathology also affects nondopaminergic areas.	(Belloli et al., 2020)	background
Starting from the knowledge that there is neurodegeneration in extranigral nuclei as part of the degenerative course of Parkinson's disease.	(Blanco-Lezcano et al., 2020)	background
Due to the fact that PD is characterized by a broad Lewy pathology localization and at the same time by a neuronal loss evident only in limited brain regions, including subsets of brain nuclei and the SN, it is indeed supposed that there	(Brazdis et al., 2020)	background

Statements	Publication	Classification
might be common features rendering PD pathology-related neurons more vulnerable.		
Not all DA neurons are equally susceptible to neurodegeneration, as DA neurons in the ventral tegmental area (VTA) are more resilient than DA neurons of the substantia nigra pars compacta (SNc).	(Buck et al., 2020) (Buck et al., 2021)	background, within
PD patients have low levels of dopamine in basal ganglia owing to a severe loss of dopaminergic neurons in the substantial nigra pars compacta (SNpc).	(Daskalaki & Tavernarakis, 2020)	background
However, while this mismatch does not at all exclude such prion like propagation of aSyn pathology, regional or cell-autonomous mechanisms, as proposed here, must also be factored into pathogenesis.	(Diederich et al., 2020)	background, within
ongoing generation of toxic dopamine metabolites in the cytosol; a greater need to buffer Ca2+ ions; and collectively, a greater degree of oxidative stress.	(El Kodsi et al., 2020)	background, within
It is estimated that 40-60% of dopaminergic neurons are already lost once motor symptoms arise, due to the degeneration of dopaminergic projections from the SNpc to the putamen in the dorsal striatum.	(Erdengiz, 2020)	Background, within
However, diagnosis usually only occurs when 40–60 % of dopaminergic neurons have already been degenerated.	(Ferreira et al., 2020)	background, within
The symptomatology is further broadened by alterations of other neuronal types throughout the course of the disease, although to a lesser extent. A9 mDA neurons, which primarily project to sensorimotor and associative striatal areas (putamen and caudate nucleus), as well as some cortical areas, are particularly vulnerable to neurodegeneration in PD. Nevertheless, despite mDA neuron neurodegeneration being the central element of PD pathology, there is also evidence of a loss of cholinergic, adrenergic, and potentially serotonergic neurons over the course of the disease, which alters cortical and basal ganglia function and has been linked to several nonmotor symptoms.	(Galet et al., 2020)	background, background, background, within
The most vulnerable neuronal subpopulations include SNpc DA neurons; cholinergic neurons in the pedunculopontine nucleus, nucleus basalis of Meynert, and dorsal motor nucleus of vagus; noradrenergic neurons in the locus coeruleus; and serotoninergic neurons in the raphe nucleus. Other proposed causes include oxidative stress (eg. loss of iron homeostasis), dopamine toxicity, autonomous pacemaking driving rhythmic Ca2+-dependent action potentials, and other vulnerabilities in mitochondrial function arising from the size of axonal arbors.	(Ge et al., 2020, p. 1)	background, within, background, within
However, the magnitude and timing of this degeneration is poorly defined because of the constraints of working with post-mortem human tissue.	(Gonzalez-Rodriguez et al., 2020)	core, background

Statements	Publication	Classification
… although it must be acknowledged that the assessment of neuronal loss has not been rigorously distinguished from phenotypic down-regulation in the vast majority of studies.		
… the PPN was chosen because it is cytologically heterogeneous and manifests both LP and cell loss in cPP. Regardless, the selective vulnerability of PPN CNs to PFFs is consistent with studies of the human cPD brain, in which neuronal cell loss and LP in the PPN is largely limited to CNs.	(Henrich et al., 2020)	background, within
However, it should be noted that few studies have performed stereological quantification of neurons in the VTA and the one study to do so reported a loss of neurons in this dopaminergic regions.	(Hijaz & Volpicelli-Daley, 2020)	core
While the clinical syndrome of PD was initially attributed to basal ganglia dysfunction, human postmortem and animal model studies have subsequently shown that non-dopaminergic neurons in other brain regions (such as vagus dorsal motor nucleus, locus coeruleus and raphe nuclei) are also involved. Although SNCA mutations are a rare cause of PD, the pivotal role of α-synuclein in the pathogenesis of PD is now clearly recognised. α-synuclein also exists in different forms/ species depending on experimental conditions, and the relative toxicity of its oligomeric and fibrillar species has been debated.	(Jankovic & Tan, 2020)	background, background, background, background
PD is known to cause progressive cell loss in various regions of the brain.	(Kao et al., 2020)	background
Moreover, cell loss has also been observed in the hypothalamus of Parkinson's disease patients; Thannickal et al. reported 50% hypothalamic cell loss in 10 patients with Parkinson's disease, with cell loss increasing with severity.	(Kubota et al., 2020)	within
Although how or why they form is unknown, aberrantly phosphorylated α-syn aggregates are found variously throughout the post-mortem brain in synucleinopathies, including most (but not all) forms of PD. Furthermore, the failure of dopamine replenishment to abate many non-motor symptoms and prevent disease progression, in concert with extranigral cell death in thalamic and cortical areas.	(Kuhlmann & Milnerwood, 2020)	background, background, within
Although the loss of DA neurons at the SNc is the canonical pathological feature in PD patients, a large body of evidence has shown that non-DA neuron populations in other regions of the brain also undergo neuron loss.	(Liang et al., 2020)	background
In late-stage Parkinson's disease, post-mortem Lewy pathology is observed in surviving nigral cells, but also throughout the brain, to varying degrees.	(MacIsaac et al., 2020)	background
Ageing, interacting with a myriad of environmental noxious factors, represents a most crucial event, linking increased inflammation and oxidative stress to mitochondrial deficits and dysregulation of lysosomal, proteosomal and autophagic functions, robustely contributing to the chronic mDAn deterioration in the PD brain.	(Marchetti, 2020)	background

Statements	Publication	Classification
A key feature of astrocyte neuroprotective properties is the activation of an antioxidant self-defense response. Indeed, DA oxidative metabolism represents a vulnerability factor linking both mitochondrial and lysosomal dysfunctions to PD pathogenesis.	(Marchetti, Leggio, et al., 2020)	within
Aging represents the most crucial event, linking increased inflammation and oxidative stress to mitochondrial dysfunction and dysregulation of lysosomal, proteosomal and autophagic functions, likely contributing to the chronic neuronal deterioration in the PD brain.	(Marchetti, Tirolo, et al., 2020)	background,
In areas such as Nucleus Basalis of Meynert (NBM), Olfactory Bulb and neurons of the enteric nerves system (ENS) no clear evidence of neuronal lose in these areas.	(Merghani, 2020)	within
These symptoms likely arise from a more widespread distribution of pathological Lewy bodies and neurites and from the degeneration of non-dopaminergic brain nuclei and are therefore treated in a patient-specific manner.	(Nolbrant, 2020)	background
One key characteristic of PD is the death of the midbrain dopaminergic (mDA) neurons, but until recently, it was impossible to study them since 60% of these neurons have disappeared by the time of diagnosis, and about 90% by the time the patients die. In contrast to mDA neurons, the effect of PD on other types of DA neurons is variable, hence their study would not be as pertinent to the elucidation of mechanisms causing PD-induced cell death. Their distinct identity is reflected in their function and leads to their unique susceptibility to death in PD, which in turn leads to the classic movement symptoms of the disease.	(Novak et al., 2020)	background, unrelated, background, background
Oxidative stress, mitochondrial dysfunction, protein aggregation and misfolding, activation of programmed cell death, neuroinflammation, neurotoxins, and the loss of neurotrophic factors (NTFs) have been proposed to be involved in neuronal loss upon PD.	(Novosadova et al., 2020)	background
Both ADHD and PD are characterized by basal ganglia alterations. Enlarged echogenic substantia nigra area has been found in children with ADHD but also in people with PD.	(Pallanti & Salerno, 2020)	background
This selective vulnerability is one hallmark for PD in patients and raises the question which cell type specific factors render SNc DaNs vulnerable to mitochondrial dysfunction.	(Paß et al., 2020)	background
PD is associated with loss of dopamine (DA) neurons in the Substantia Nigra pars compacta (SNpc).	(Salado-Manzano et al., 2020)	background
Studies have also reported a substantial loss of cholinergic neurons in the different brain regions and also serotonergic neurons of the raphe nuclei in PD pathogenesis.	(Sengupta et al., 2020)	background

Statements	Publication	Classification
… likely play an important role in DAergic neuroprotection against oxidative damage.	(Serapide et al., 2020)	background, within
Even though the PD is depicted by the loss of nigrostriatal dopamine (DA) neurons, but its pathology also involves the profound loss of serotonergic and cholinergic neurons as well as a profound loss of neurons in LC.	(Singh, 2020)	background, within
PD is characterized by progressive dopaminergic cell death in the SNc, which primarily projects to the basal ganglia, and a less severe but significant loss of dopaminergic neurons in the VTA.	(Swanson et al., 2020)	background
Besides the SNc DA neurons, TH positive noradrenergic cells in the locus coeruleus (lc), tryptophan hydroxylase 2 (TH2) -positive serotonergic cells in the raphe nuclei (rn) and choline acetyltransferase (ChAT)-positive cholinergic neurons in the nucleus basalis of Meynert (nbM) have been reported to exhibit LBP.	(Tan, 2020)	background
In addition to SNc DA neurons, a number of additional cell types with a distinct neurochemical profile may be affected in PD, including noradrenergic cells in the locus coeruleus (lc), serotonergic cells in the raphe nuclei (rn) and cholinergic neurons of the nucleus basalis of Meynert (nbM).	(Tan et al., 2020)	background, within
The presence of LBs in the nucleus basilaris was declared pathognomonic to PD; however, they were detected in 100% of patients diagnosed with idiopathic PD by ubiquitin immunohistochemistry.	(Tanaka et al., 2020)	background, unrelated
However, there is also widespread cell loss in several subcortical nuclei, including the locus coeruleus (LC).	(Tyer & Hill, 2020)	background, within
The average reduction of nigral DA neurons determined by stereological estimates in 181 PD patients across 12 studies has been estimated to around ~68% but reflected considerable inter-study and inter-individual variation.	(von Linstow et al., 2020)	background, within
The most important of these is likely to be the neuron's massive axonal arbor. While transmitter phenotype may not be a universally shared trait of vulnerable neurons, other traits are shared. If Ca2+ and feedforward control of mitochondrial OXPHOS are the keys to the vulnerability of SNc DAergic neurons in PD, other neuronal populations at-risk in PD should have a similar phenotype. Many other neurons, particularly in the brainstem, are vulnerable in PD.	(Zampese & Surmeier, 2020)	background, background, background
PD is classically defined by loss of dopaminergic neurons within the substantia nigra pars compacta, but evidence of neuronal cell death within other brains regions and diffuse Lewy body pathology, including in cortical regions, is commonly observed in the disease.	(Anderson et al., 2021)	background
We chose to analyze the amygdala because it is a brain area severely affected by α-syn pathology in patients with Braak stages IV to VI without the presence of overt neurodegeneration.	(Apicco et al., 2021)	background, within

Statements	Publication	Classification
By the time PD can be diagnosed on the basis of its cardinal motor symptoms, the neurodegeneration not only in the substantia nigra pars compacta but more widely — has been advancing for many years. In a similar theory of susceptibility, degeneration in the substantia nigra and other nuclei is thought to be accelerated, perhaps owing to high energy demands as a result of high baseline activity and arborization.	(Berg et al., 2021)	background, background
The extensive axonal arborization of dopaminergic nigrostriatal neurons may make these cells especially vulnerable to transport deficits.	(Boecker et al., 2021)	background
… it is also unclear why certain types of neurons appear to be much more vulnerable than others.	(Borghammer, 2021)	background
Parkinson's disease (PD) is characterized by a loss of substantia nigra pars compacta (SNc) dopaminergic neurons resulting in motor disturbances.	(Dwyer et al., 2021)	background
VTA dopamine neurons, which are far less vulnerable to degeneration, did not show altered survival in the absence of parkin, suggesting that the absence of parkin is potentially lethal only in neurons that exhibit higher energetic demands and higher oxidative stress associated with profuse arborization.	(Fehr, 2021)	background
Likewise, accumulation of iron in the brain, especially in the basal ganglia, is often more susceptible to neurodegenerative diseases, suggests its potential involvement.	(Fidelis et al., 2021)	background, within
The neuronal loss in the PD brain is not restricted to substantia nigra only, but catecholaminergic as well as non-catecholaminergic neurons in the dorsal motor nucleus of the vagus, olfactory bulb, ventral tegmental region, locus coeruleus, raphe nucleus, and nucleus basalis of Meynert, and the neuronal loss is associated with reactive gliosis.	(Ganguly et al., 2021)	background, within, core
A recent review on cell loss throughout the PD brain took great care to analyze all the studies (ranging from 1953 to 2015) demonstrating cell loss and classified whether these studies utilized unbiased stereology to count neuron populations within brain nuclei. Overall, clearly, there is cell loss in brain areas in addition to the SNc, but neuron loss in these other brain areas requires further validation. It is also important to consider that while the cells might not die in PD, they could be but how these other neurons and circuits contribute to PD symptoms, and in which circuits synaptic dysfunction occurs remains unclear.	(Gcwensa et al., 2021)	core, core
The degeneration of dopaminergic neurons and the decrease in dopamine content are main causes of Parkinson's disease and its associated dyskinesia.	(Han et al., 2021)	background
Cellular mechanisms such as impaired protein clearance, altered mitochondrial function, inflammation, and management of reactive oxygen species have all been proposed to play a role in cell death in Parkinson's disease.	(Helmich & Lehéricy, 2021)	background

Statements	Publication	Classification
These multifaceted clinical features correlate with a sequential degeneration of neurons within several discrete loci of the brain, which points to both spreading neuropathology and regional vulnerability as underlying causes for PD.	(Huntington & Srinivasan, 2021)	background
Considering that clinical signs of PD are presented when about more than 50% of DA neurons in the SNc and 70% of their synaptic terminals in the striatum are lost …	(Hur & Lee, 2021)	background
There are nine quantitative postmortem studies published assessing the loss of A6 neurons in PD that range from very little to 95% loss with greater disease stages and longer disease durations. Selectively vulnerable brain regions to LP include (from caudal to rostral); the noradrenergic A6 locus coeruleus, the midbrain dopaminergic regions, and the basal forebrain cholinergic regions. Assessments of the SNc are well established, with an average 68% loss of neurons using stereology, and more severe loss with increasing disease duration and severity. Publications on the A10 regions are scarce with an average 31% loss, and publications on the basal forebrain vary greatly in their outcome (up to 72% loss). Cell loss in these later regions are both impacted by Alzheimer pathology, a variable that has not often been controlled for.	(Huynh et al., 2021)	core
The basic tenet of this viewpoint is that certain neuronal populations, due to their inherent cellular and network properties, are more vulnerable to α-syn induced proteopathic stress and susceptible to changes in the local microenvironment (e.g., neuroinflammation, metabolic deficits).	(Jan et al., 2021, p.)	background, unrelated
PD is a multifactorial neurodegenerative disease that is believed to be caused by both genetic changes and environmental factors. It is characterized by the deposition of toxic α-synuclein inclusions that lead to the death of dopaminergic neurons in the striatum and, consequently, motor dysfunction.	(Jang et al., 2021)	background
PD is one of the most common neurodegenerative disorders with cardinal signs of resting tremor, bradykinesia and rigidity, which mostly result from prominent death of dopaminergic (DA) neurons in the substantia nigra (SN) pars compacta. Since the disease discovery over 200 years ago, there has been no disease-modifying treatment for PD.	(Kamienieva et al., 2021)	background, background
A more widespread neurodegeneration affecting non-dopaminergic systems has also been described. Alterations in neurotransmitter levels have been detected in the nucleus basalis of Meynert (acetylcholine), locus coeruleus (noradrenaline) and the raphe nuclei (serotonin), which may contribute to the non-motor components of the illness including cognition, depression and dementia.	(Kee & Papathanou, 2021)	background, unrelated
Future versions of the model should take into account the following. (i) Effect of a complex structure of axonal arbor. DA neurons in SNc have hundreds of thousands of synapses, which is two orders of magnitude more than other neurons in basal ganglia.	(Kuznetsov & Kuznetsov, 2021)	background

Statements	Publication	Classification
Moreover, the higher pacemaker activity and massive axonal arborization of Tg2576 DA neurons could contribute to their vulnerability, because of the high metabolic demand and need of increased mitochondrial efficiency.	(La Barbera et al., 2021)	background
Interestingly, these SNc-ALDH1A1+neurons have been shown to undergo degeneration in PD and have been linked directly with the motor impairments associated with PD.	(Lerner et al., 2021)	background
Characterized by intracellular aggregation of alpha synuclein (α-syn) and loss of dopaminergic neurons, PD affects the substantia nigra pars compacta (SNpc), critical for motor function, and other nuclei.	(McQuade et al., 2021)	background
Pathologically, PD is associated with Lewy bodies and Lewy neurites consisting of misfolded and aggregated alpha-synuclein protein. Physiologically, PD results in loss of dopamine neurons in the substantia nigra pars compacta and subsequent basal ganglia dysfunction.	(Mitchell & Sidiropoulos, 2021)	background
Since the metabolic demands of SNc neurons are particularly high when compared to any other neuronal types including neurons of other dopaminergic systems.	(Muddapu & Chakravarthy, 2021)	background, within
With the progressive loss of dopaminergic and non-dopaminergic neurons and synapses, available therapies gradually become less effective at controlling PD motor symptoms.	(Pagano et al., 2021)	background, unrelated
The pathology of PD involves the loss of dopamine (DA) neurons of the substantia nigra pars compacta, but other lesions in the form of Lewy bodies (aggregates of α-synuclein) and neurites have been reported in multiple regions of the central and peripheral nervous system, including the locus coeruleus, the dorsal raphe nucleus and the dorsal motor nucleus of the vagus.	(Perlikowska, 2021)	background, unrelated
…which insinuated that KORs and MORs are less sensitive to brain injury then DORs and stimulation of DORs might display a protective effect.		
While dopaminergic neuronal death is a key pathological hallmark of PD …	(Pinson et al., 2021)	background
Given that the GABAergic neurons in the striatum and SNr are less affected by neuronal dysfunction and loss in PD than the dopaminergic neurons of the SNc …	(Ryan et al., 2021)	background, contrary
Emerging animal PD models and clinical PD studies clearly show loss of these three catecholaminergic neuronal populations contributing to disease symptom characteristics.	(Salvatore et al., 2021)	background
Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Its motor symptoms are characterized by progressive degeneration of dopamine (DA)-releasing neurons in the substantia nigra (SN), while	(Siller et al., 2021)	background, contrary

Statements	Publication	Classification
neighboring DA neurons in the ventral tegmental area (VTA) remain largely unaffected.		
Classically, PD is considered a motor disorder driven by dopamine system impairment, but the importance of non-motor symptoms and pathophysiological mechanisms involving different brain regions, neuronal populations, and signaling pathways has been recently recognized.	(Stanojlovic et al., 2021)	background
In spite of this widespread dysfunction across multiple neurologic systems, the SNpc clearly manifests an unparalleled degree of neuronal loss in the PD brain.	(Subrahmanian & LaVoie, 2021)	background, within
PFF uptake's activity dependence is a fundamental insight into how αSYN pathology might spread in the human brain. It is of considerable interest that most of the brain neurons that manifest LP in PD are spontaneously (or autonomously) active neurons with massive axonal arbors.	(Surmeier, 2021)	background
Mounting evidence suggests that the maintenance of proper NAD+ homeostasis is essential for DA neurons that are special types of neurons differing from most of the other neurons in the central nervous system in several ways, including greater energy demand, increased mitochondrial activity, reactive oxygen species release, and altered Ca+ homeostasis.	(Turconi et al., 2021)	background
Several stereological studies have verified atrophy and neurodegeneration in different areas in the postmortem brain of patients with PD.	(Villar-Conde et al., 2021)	background
And the striatum is not considered the brain region where neuron loss occurs in PD.	(Yang et al., 2021)	unrelated
The distribution of α-synuclein in the cranial nervous system and the site where neuronal cell loss occurs in PD have been investigated.	(Yoshino et al., 2021)	background
This may explain the increased vulnerability of dopaminergic neurons to degeneration in Parkinson's disease, and why treatment with dopamine precursor, L-dopa, leads to increased dyskinesia follow long-term treatment.	(Young, 2021)	background
Loss of dopaminergic neurons in the brainstem, including the substantia nigra pars compacta (SNpc), ventral tegmental area (VTA), and locus coeruleus (LC), is characteristic of Parkinson's disease (PD).	(Ishikuro et al., 2021)	background, within

Table 6 Statements citing our review — classified according to the relationship to the core message

12.04 Bibliography for Table 6

Alegre-Cortés, E., Muriel-González, A., Canales-Cortés, S., Uribe-Carretero, E., Martínez-Chacón, G., Aiastui, A., López de Munain, A., Niso-Santano, M., Gonzalez-Polo, R. A., & Fuentes, J. M. (2020). Toxicity of necrostatin-1 in Parkinson's disease models. Antioxidants, 9(6), 524. https://doi.org/10.3390/antiox9060524

Anderson, F. L., von Herrmann, K. M., Young, A. L., & Havrda, M. C. (2021). Bbc3 loss enhances survival and protein clearance in neurons exposed to the organophosphate pesticide chlorpyrifos. Toxicological Sciences. https://doi.org/10.1093/toxsci/kfab090

Apicco, D. J., Shlevkov, E., Nezich, C. L., Tran, D. T., Guilmette, E., Nicholatos, J. W., Bantle, C. M., Chen, Y., Glajch, K. E., & Abraham, N. A. (2021). The Parkinson's disease-associated gene ITPKB protects against α-synuclein aggregation by regulating ER-to-mitochondria calcium release. Proceedings of the National Academy of Sciences, 118(1). https://doi.org/10.1073/pnas.2006476118

Aryal, S., Skinner, T., Bridges, B., & Weber, J. T. (2020). The Pathology of Parkinson's Disease and Potential Benefit of Dietary Polyphenols. Molecules, 25(19), 4382. https://doi.org/10.3390/molecules25194382

Assini, R. (2019). Extrastriatal GABA A receptors as a nondopaminergic target in the treatment of motor symptoms of Parkinson's disease and levodopa-induced dyskinesia [PhD Thesis]. Rutgers University-Graduate School-Newark.

Bastasic, J. (2019). The Impact of Parkinson's Disease on Mammalian Adult Neurogenesis.

Belloli, S., Morari, M., Murtaj, V., Valtorta, S., Moresco, R. M., & Gilardi, M. C. (2020). Translation imaging in Parkinson's Disease: Focus on neuroinflammation. Frontiers in Aging Neuroscience, 12, 152. https://doi.org/10.3389/fnagi.2020.00152

Benkert, J., Hess, S., Roy, S., Beccano-Kelly, D., Wiederspohn, N., Duda, J., Simons, C., Patil, K., Gaifullina, A., Mannal, N., Dragicevic, E., Spaich, D., Müller, S., Nemeth, J., Hollmann, H., Deuter, N., Mousba, Y., Kubisch, C., Poetschke, C., … Liss, B. (2019). Cav2.3 channels contribute to dopaminergic neuron loss in a model of Parkinson's disease. Nature Communications, 10(1), 5094. https://doi.org/10.1038/s41467-019-12834-x

Berg, D., Borghammer, P., Fereshtehnejad, S.-M., Heinzel, S., Horsager, J., Schaeffer, E., & Postuma, R. B. (2021). Prodromal Parkinson disease subtypes—Key to understanding heterogeneity. Nature Reviews Neurology, 17(6), 349–361. https://doi.org/10.1038/s41582-021-00486-9

Betts, M. J., Kirilina, E., Otaduy, M. C., Ivanov, D., Acosta-Cabronero, J., Callaghan, M. F., Lambert, C., Cardenas-Blanco, A., Pine, K., & Passamonti, L. (2019). Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases. Brain, 142(9), 2558–2571. https://doi.org/10.1093/brain/awz193

Blair, J. C., Barrett, M. J., Patrie, J., Flanigan, J. L., Sperling, S. A., Elias, W. J., & Druzgal, T. J. (2019). Brain MRI reveals ascending atrophy in Parkinson's disease across severity. Frontiers in Neurology, 10, 1329. https://doi.org/10.3389/fneur.2019.01329

Blanco-Lezcano, L., Alberti Amador, E., González Fraguela, M. E., Larrea, G. Z. L. de, Pérez Serrano, R. M., Jiménez Luna, N. A., Camejo Rodríguez, D., Serrano Sánchez, T., Francis Turner, L., & Estupiñán Díaz, B. (2020). Motor Coordination Disorders Evaluated through the Grid Test and

Changes in the Nigral Nrf2 mRNA Expression in Rats with Pedunculopontine Lesion. Behavioral Sciences, 10(10), 156. https://doi.org/10.3390/bs10100156

Blesa, J., & Vila, M. (2019). Parkinson disease, substantia nigra vulnerability, and calbindin expression: Enlightening the darkness? Wiley Online Library.

Boecker, C. A., Goldsmith, J., Dou, D., Cajka, G. G., & Holzbaur, E. L. (2021). Increased LRRK2 kinase activity alters neuronal autophagy by disrupting the axonal transport of autophagosomes. Current Biology, 31(10), 2140–2154. https://doi.org/10.1016/j.cub.2021.02.061

Borghammer, P. (2021). The α-Synuclein Origin and Connectome Model (SOC Model) of Parkinson's Disease: Explaining Motor Asymmetry, Non-Motor Phenotypes, and Cognitive Decline. Journal of Parkinson's Disease, 11(2), 455. https://doi.org/10.3233/JPD-202481

Brazdis, R.-M., Alecu, J. E., Marsch, D., Dahms, A., Simmnacher, K., Lörentz, S., Brendler, A., Schneider, Y., Marxreiter, F., & Roybon, L. (2020). Demonstration of brain region-specific neuronal vulnerability in human iPSC-based model of familial Parkinson's disease. Human Molecular Genetics, 29(7), 1180–1191. https://doi.org/10.1093/hmg/ddaa039

Bucher, M. L. (2019). Vesicular Sequestration of Dopamine Modulates Neuronal Health in Parkinson's Disease Models [PhD Thesis]. University of Pittsburgh.

Buck, S. A., Steinkellner, T., Aslanoglou, D., Villeneuve, M., Bhatte, S. H., Childers, V. C., Rubin, S. A., De Miranda, B. R., O'Leary, E. I., & Neureiter, E. G. (2020). VGLUT modulates sex differences in dopamine neuron vulnerability to age-related neurodegeneration. BioRxiv. https://doi.org/10.1101/2020.11.11.379008

Buck, S. A., Steinkellner, T., Aslanoglou, D., Villeneuve, M., Bhatte, S. H., Childers, V. C., Rubin, S. A., De Miranda, B. R., O'Leary, E. I., & Neureiter, E. G. (2021). Vesicular glutamate transporter modulates sex differences in dopamine neuron vulnerability to age-related neurodegeneration. Aging Cell, 20(5), e13365. https://doi.org/10.1111/acel.13365

Daskalaki, I., & Tavernarakis, N. (2020). Mitochondrial biogenesis in organismal senescence and neurodegeneration. Mechanisms of Ageing and Development, 191, 111345. https://doi.org/10.1016/j.mad.2020.111345

Deal, S. L., & Yamamoto, S. (2019). Unraveling novel mechanisms of neurodegeneration through a large-scale forward genetic screen in Drosophila. Frontiers in Genetics, 9, 700. https://doi.org/10.3389/fgene.2018.00700

Díaz, M., Luis-Amaro, A. C., Rodriguez Barreto, D., Casañas-Sánchez, V., Pérez, J. A., & Marin, R. (2019). Lipostatic mechanisms preserving cerebellar lipids in MPTP-treated mice: Focus on membrane microdomains and lipid-related gene expression. Frontiers in Molecular Neuroscience, 12, 93. https://doi.org/10.3389/fnmol.2019.00093

Diederich, N. J., Uchihara, T., Grillner, S., & Goetz, C. G. (2020). The Evolution-Driven Signature of Parkinson's Disease. Trends in Neurosciences, 43(7), 475–492. https://doi.org/10.1016/j.tins.2020.05.001

Dwyer, Z. (2019). Environmental toxicant exposure and Parkinson's disease: LRRK2 and inflammatory processes [PhD Thesis]. Carleton University.

Dwyer, Z., Chaiquin, M., Landrigan, J., Ayoub, K., Shail, P., Rocha, J., Childers, C. L., Storey, K. B., Philpott, D. J., & Sun, H. (2021). The impact of dextran sodium sulphate and probiotic pre-treatment in a murine model of Parkinson's disease. Journal of Neuroinflammation, 18(1), 1–15. https://doi.org/10.1186/s12974-020-02062-2

El Kodsi, D. N., Tokarew, J. M., Sengupta, R., Lengacher, N. A., Ng, A. C., Boston, H., Jiang, Q., Palmberg, C., Pileggi, C., & Shutinoski, B. (2020). Parkinson disease-linked parkin mediates redox reactions that lower oxidative stress in mammalian brain. BioRxiv. https://doi.org/10.1101/2020.04.26.062380

Erdengiz, D. (2020). Investigation of Alpha-Synuclein Induced Disruption of Calcium Homeostasis in Synucleinopathies [PhD Thesis]. Georgetown University.

Fehr, T. (2021). Characterizing Cysteine Modifications in Parkinson's-Linked Parkin and Their Influence on Dopaminergic Cell Health [PhD Thesis]. Université d'Ottawa/University of Ottawa.

Ferreira, A. F. F., Binda, K. H., Singulani, M. P., Pereira, C. P. M., Ferrari, G. D., Alberici, L. C., Real, C. C., & Britto, L. R. (2020). Physical exercise protects against mitochondria alterations in the 6-hidroxydopamine rat model of Parkinson's disease. Behavioural Brain Research, 387, 112607. https://doi.org/10.1016/j.bbr.2020.112607

Fidelis, K. R., dos Santos Nunes, R. G., da Silva, C. S., Oliveira, C. V. B., Costa, A. R., de Lima Silva, J. R., Dos Santos, L. B., de Oliveira, E. E. S., Pereira, P. S., & de Menezes, I. R. A. (2021). Evaluation of the neuroprotective effect of rutin on Drosophila melanogaster about behavioral and biochemical aspects induced by mercury chloride (HgCl2). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 109119. https://doi.org/10.1016/j.cbpc.2021.109119

Galet, B., Cheval, H., & Ravassard, P. (2020). Patient-derived midbrain organoids to explore the molecular basis of Parkinson's disease. Frontiers in Neurology, 11, 1005. https://doi.org/10.3389/fneur.2020.01005

Ganguly, U., Singh, S., Pal, S., Prasad, S., Agrawal, B. K., Saini, R. V., & Chakrabarti, S. (2021). Alpha-Synuclein as a Biomarker of Parkinson's Disease: Good, but Not Good Enough. Frontiers in Aging Neuroscience, 365. https://doi.org/10.3389/fnagi.2021.702639

Gcwensa, N. Z., Russell, D. L., Cowell, R. M., & Volpicelli-Daley, L. A. (2021). Molecular Mechanisms Underlying Synaptic and Axon Degeneration in Parkinson's Disease. Frontiers in Cellular Neuroscience, 15, 44. https://doi.org/10.3389/fncel.2021.626128

Ge, P., Dawson, V. L., & Dawson, T. M. (2020). PINK1 and Parkin mitochondrial quality control: A source of regional vulnerability in Parkinson's disease. Molecular Neurodegeneration, 15(1), 1–18. https://doi.org/10.1186/s13024-020-00367-7

Giannopoulos, S., Samardzic, K., Raymond, B. B., Djordjevic, S. P., & Rodgers, K. J. (2019). L-DOPA causes mitochondrial dysfunction in vitro: A novel mechanism of L-DOPA toxicity uncovered. The International Journal of Biochemistry & Cell Biology, 117, 105624. https://doi.org/10.1016/j.biocel.2019.105624

Giguère, N., Delignat-Lavaud, B., Herborg, F., Voisin, A., Li, Y., Jacquemet, V., Anand-Srivastava, M., Gether, U., Giros, B., & Trudeau, L.-É. (2019). Increased vulnerability of nigral dopamine neurons after expansion of their axonal arborization size through D2 dopamine receptor conditional knockout. PLOS Genetics, 15(8), e1008352. https://doi.org/10.1371/journal.pgen.1008352

Goh, S. Y., Chao, Y. X., Dheen, S. T., Tan, E.-K., & Tay, S. S.-W. (2019). Role of MicroRNAs in Parkinson's disease. International Journal of Molecular Sciences, 20(22), 5649. https://doi.org/10.3390/ijms20225649

Gonzalez-Rodriguez, P., Zampese, E., & Surmeier, D. J. (2020). Selective neuronal vulnerability in Parkinson's disease. In Progress in brain research (Vol. 252, pp. 61–89). Elsevier.

Han, Y., Wang, T., Li, C., Wang, Z., Zhao, Y., He, J., Fu, L., & Han, B. (2021). Ginsenoside Rg3 exerts a neuroprotective effect in rotenone-induced Parkinson's disease mice via its anti-oxidative properties. European Journal of Pharmacology, 174413. https://doi.org/10.1016/j.ejphar.2021.174413

Helmich, R. C., & Lehéricy, S. (2021). Dying-back of ascending noradrenergic projections in Parkinson's disease. Brain. https://doi.org/10.1093/brain/awab286

Henrich, M. T., Geibl, F. F., Lakshminarasimhan, H., Stegmann, A., Giasson, B. I., Mao, X., Dawson, V. L., Dawson, T. M., Oertel, W. H., & Surmeier, D. J. (2020). Determinants of seeding and spreading of α-synuclein pathology in the brain. Science Advances, 6(46), eabc2487. https://doi.org/10.1126/sciadv.abc2487

Hernandez, L. F., Obeso, I., Costa, R. M., Redgrave, P., & Obeso, J. A. (2019). Dopaminergic Vulnerability in Parkinson Disease: The Cost of Humans' Habitual Performance. Trends in Neurosciences, 42(6), 375–383. https://doi.org/10.1016/j.tins.2019.03.007

Hernandez-Baltazar, D., Nadella, R., Mireya Zavala-Flores, L., Rosas-Jarquin, C. de J., Rovirosa-Hernandez, M. de J., & Villanueva-Olivo, A. (2019). Four main therapeutic keys for Parkinson's disease: A mini review. Iranian Journal of Basic Medical Sciences, 22(7), 716–721. https://doi.org/10.22038/ijbms.2019.33659.8025

Hijaz, B. A., & Volpicelli-Daley, L. A. (2020). Initiation and propagation of α-synuclein aggregation in the nervous system. Molecular Neurodegeneration, 15(1), 1–12. https://doi.org/10.1186/s13024-020-00368-6

Huntington, T. E., & Srinivasan, R. (2021). Adeno-Associated Virus Expression of α-Synuclein as a Tool to Model Parkinson's Disease: Current Understanding and Knowledge Gaps. Aging and Disease, 0. https://doi.org/10.14336/AD.2021.0517

Hur, E.-M., & Lee, B. D. (2021). LRRK2 at the Crossroad of Aging and Parkinson's Disease. Genes, 12(4), 505. https://doi.org/10.3390/genes12040505

Huynh, B., Fu, Y., Kirik, D., Shine, J. M., & Halliday, G. M. (2021). Comparison of Locus Coeruleus Pathology with Nigral and Forebrain Pathology in Parkinson's Disease. Movement Disorders. https://doi.org/10.1002/mds.28615

Ishikuro, K., Hattori, N., Imanishi, R., Furuya, K., Nakata, T., Dougu, N., Yamamoto, M., Konishi, H., Nukui, T., & Hayashi, T. (2021). A Parkinson's disease patient displaying increased neuromelanin-sensitive areas in the substantia nigra after rehabilitation with tDCS: A case report. Neurocase, 1–8. https://doi.org/10/gmwf55

Jan, A., Gonçalves, N. P., Vaegter, C. B., Jensen, P. H., & Ferreira, N. (2021). The Prion-Like Spreading of Alpha-Synuclein in Parkinson's Disease: Update on Models and Hypotheses. International Journal of Molecular Sciences, 22(15), 8338. https://doi.org/10.3390/ijms22158338

Jang, C. H., Oh, J., Lim, J. S., Kim, H. J., & Kim, J.-S. (2021). Fermented soy products: Beneficial potential in neurodegenerative diseases. Foods, 10(3), 636. https://doi.org/10.3390/foods10030636

Jankovic, J., & Tan, E. K. (2020). Parkinson's disease: Etiopathogenesis and treatment. Journal of Neurology, Neurosurgery & Psychiatry, 91(8), 795–808. https://doi.org/10.1136/jnnp-2019-322338

Jellinger, K. A. (2019). Neuropathology and pathogenesis of extrapyramidal movement disorders: A critical update—I. Hypokinetic-rigid movement disorders. Journal of Neural Transmission, 126(8), 933–995.

Kamienieva, I., Duszyński, J., & Szczepanowska, J. (2021). Multitasking guardian of mitochondrial quality: Parkin function and Parkinson's disease. Translational Neurodegeneration, 10(1), 1–18. https://doi.org/10.1186/s40035-020-00229-8

Kao, C.-Y., Xu, M., Wang, L., Lin, S.-C., Lee, H.-J., Duraine, L., Bellen, H. J., Goldstein, D. S., Tsai, S. Y., & Tsai, M.-J. (2020). Elevated COUP-TFII expression in dopaminergic neurons accelerates the progression of Parkinson's disease through mitochondrial dysfunction. PLoS Genetics, 16(6), e1008868. https://doi.org/10.1371/journal.pgen.1008868

Kee, N., & Papathanou, M. (2021). Single-Cell Technologies in Parkinson′ s Disease. https://doi.org/10.1016/B978-0-12-801238-3.11613-7

Korecka, J. A., Thomas, R., Christensen, D. P., Hinrich, A. J., Ferrari, E. J., Levy, S. A., Hastings, M. L., Hallett, P. J., & Isacson, O. (2019). Mitochondrial clearance and maturation of autophagosomes are compromised in LRRK2 G2019S familial Parkinson's disease patient fibroblasts. Human Molecular Genetics, 28(19), 3232–3243. https://doi.org/10.1093/hmg/ddz126

Kouli, A. (2019). The neuroinflammatory basis of disease progression and cognitive impairment in Parkinson's disease [PhD Thesis]. University of Cambridge.

Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018). Parkinson's disease: Etiology, neuropathology, and pathogenesis. Exon Publications, 3–26. https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1

Kubota, M., Kobayashi, N., Sugizaki, T., Shimoda, M., Kawahara, M., & Tanaka, K. (2020). Carnosine suppresses neuronal cell death and inflammation induced by 6-hydroxydopamine in an in vitro model of Parkinson's disease. PLoS One, 15(10), e0240448. https://doi.org/10.1371/journal.pone.0240448

Kuhlmann, N., & Milnerwood, A. J. (2020). A critical LRRK at the synapse? The neurobiological function and pathophysiological dysfunction of LRRK2. Frontiers in Molecular Neuroscience, 13, 153. https://doi.org/10.3389/fnmol.2020.00153

Kuznetsov, I. A., & Kuznetsov, A. V. (2019). Modelling transport and mean age of dense core vesicles in large axonal arbours. Proceedings of the Royal Society A, 475(2228), 20190284. https://doi.org/10.1098/rspa.2019.0284

Kuznetsov, I. A., & Kuznetsov, A. V. (2021). Can the lack of fibrillar form of alpha-synuclein in Lewy bodies be explained by its catalytic activity? BioRxiv. https://doi.org/10.1101/2021.05.09.443304

La Barbera, L., Vedele, F., Nobili, A., Krashia, P., Spoleti, E., Latagliata, E. C., Cutuli, D., Cauzzi, E., Marino, R., & Viscomi, M. T. (2021). Nilotinib restores memory function by preventing dopaminergic neuron degeneration in a mouse model of Alzheimer's Disease. Progress in Neurobiology, 202, 102031. https://doi.org/10.1016/j.pneurobio.2021.102031

Lerner, S., Eilam, R., Adler, L., Baruteau, J., Kreiser, T., Tsoory, M., Brandis, A., Mehlman, T., Ryten, M., & Botia, J. A. (2021). ASL expression in ALDH1A1+ neurons in the substantia nigra metabolically contributes to neurodegenerative phenotype. Human Genetics, 1–15. https://doi.org/10.1007/s00439-021-02345-5

Liang, J. J., McKinnon, I. A., & Rankin, C. H. (2020). The contribution of C. elegans neurogenetics to understanding neurodegenerative diseases. Journal of Neurogenetics, 34(3–4), 527–548. https://doi.org/10.1080/01677063.2020.1803302

Liss, B., & Striessnig, J. (2019). The potential of L-type calcium channels as a drug target for neuroprotective therapy in Parkinson's disease. Annual Review of Pharmacology and Toxicology, 59, 263–289. https://doi.org/10.1146/annurev-pharmtox-010818-021214

MacIsaac, S., Quevedo Melo, T., Zhang, Y., Volta, M., Farrer, M. J., & Milnerwood, A. J. (2020). Neuron-autonomous susceptibility to induced synuclein aggregation is exacerbated by endogenous Lrrk2 mutations and ameliorated by Lrrk2 genetic knock-out. Brain Communications, 2(1), fcz052. https://doi.org/10.1093/braincomms/fcz052

Marchetti, B. (2018). Wnt/β-catenin signaling pathway governs a full program for dopaminergic neuron survival, neurorescue and regeneration in the MPTP mouse model of Parkinson's disease. International Journal of Molecular Sciences, 19(12), 3743. https://doi.org/10.3390/ijms19123743

Marchetti, B. (2020). Nrf2/Wnt resilience orchestrates rejuvenation of glia-neuron dialogue in Parkinson's disease. Redox Biology, 101664. https://doi.org/10.1016/j.redox.2020.101664

Marchetti, B., Leggio, L., L'Episcopo, F., Vivarelli, S., Tirolo, C., Paternò, G., Giachino, C., Caniglia, S., Serapide, M. F., & Iraci, N. (2020). Glia-derived extracellular vesicles in Parkinson's disease. Journal of Clinical Medicine, 9(6), 1941. https://doi.org/10.3390/jcm9061941

Marchetti, B., Tirolo, C., L'Episcopo, F., Caniglia, S., Testa, N., Smith, J. A., Pluchino, S., & Serapide, M. F. (2020). Parkinson's disease, aging and adult neurogenesis: Wnt/β-catenin signalling as the key to unlock the mystery of endogenous brain repair. Aging Cell, 19(3), e13101. https://doi.org/10.1111/acel.13101

McQuade, R. M., Singleton, L. M., Wu, H., Lee, S., Constable, R., Di Natale, M., Ringuet, M. T., Berger, J. P., Kauhausen, J., & Parish, C. L. (2021). The association of enteric neuropathy with gut phenotypes in acute and progressive models of Parkinson's disease. Scientific Reports, 11(1), 1–13. https://doi.org/10.1038/s41598-021-86917-5

Merghani, M. M. (2020). EFFECT OF MPTP ON α-SYNUCLEIN SPREADING, ACCUMULATION AND TOXICITY IN MICE WITH INTRASTRIATAL INNOCULATION OF HUMAN α-SYNUCLEIN PREFORMED FIBRIL. https://scholarworks.uaeu.ac.ae/all_theses/797/

Mitchell, S. D., & Sidiropoulos, C. (2021). Therapeutic Applications of Botulinum Neurotoxin for Autonomic Symptoms in Parkinson's Disease: An Updated Review. Toxins, 13(3), 226. https://doi.org/10.3390/toxins13030226

Monzani, E., Nicolis, S., Dell'Acqua, S., Capucciati, A., Bacchella, C., Zucca, F. A., Mosharov, E. V., Sulzer, D., Zecca, L., & Casella, L. (2019). Dopamine, oxidative stress and protein–quinone modifications in Parkinson's and other neurodegenerative diseases. Angewandte Chemie International Edition, 58(20), 6512–6527. https://doi.org/10.1002/anie.201811122

Muddapu, V. R., & Chakravarthy, V. S. (2021). Influence of energy deficiency on the subcellular processes of Substantia Nigra Pars Compacta cell for understanding Parkinsonian neurodegeneration. Scientific Reports, 11(1), 1–36. https://doi.org/10.1038/s41598-021-81185-9

Muddapu, V. R., Mandali, A., Chakravarthy, V. S., & Ramaswamy, S. (2019). A computational model of loss of dopaminergic cells in Parkinson's disease due to glutamate-induced excitotoxicity. Frontiers in Neural Circuits, 13, 11. https://doi.org/10.3389/fncir.2019.00011

Nissen, S. K., Shrivastava, K., Schulte, C., Otzen, D. E., Goldeck, D., Berg, D., Møller, H. J., Maetzler, W., & Romero-Ramos, M. (2019). Alterations in blood monocyte functions in Parkinson's disease. Movement Disorders, 34(11), 1711–1721. https://doi.org/10.1002/mds.27815

Nolbrant, S. (2020). Directing and Dissecting the Fate of Dopaminergic Neurons-Multiple Avenues towards Cell Replacement Therapy in Parkinson's Disease [PhD Thesis]. Lund University.

Novak, G., Kyriakis, D., Grzyb, K., Bernini, M., Finkbeiner, S., & Skupin, A. (2020). Single cell transcriptomics of human PINK1 iPSC differentiation dynamics reveal a core network of Parkinson's disease. https://doi.org/10.21203/rs.3.rs-72882/v1

Novosadova, E. V., Nenasheva, V. V., Makarova, I. V., Dolotov, O. V., Inozemtseva, L. S., Arsenyeva, E. L., Chernyshenko, S. V., Sultanov, R. I., Illarioshkin, S. N., & Grivennikov, I. A. (2020). Parkinson's Disease–Associated Changes in the Expression of Neurotrophic Factors and their Receptors upon Neuronal Differentiation of Human Induced Pluripotent Stem Cells. Journal of Molecular Neuroscience, 70(4), 514–521. https://doi.org/10.1007/s12031-019-01450-5

Oertel, W. H., Henrich, M. T., Janzen, A., & Geibl, F. F. (2019). The locus coeruleus: Another vulnerability target in Parkinson's disease. Movement Disorders, 34(10), 1423–1429. https://doi.org/10.1002/mds.27785

Pagano, G., Boess, F. G., Taylor, K. I., Ricci, B., Mollenhauer, B., Poewe, W., Boulay, A., Anzures-Cabrera, J., Vogt, A., & Marchesi, M. (2021). A Phase II study to evaluate the safety and efficacy of prasinezumab in early Parkinson's disease (PASADENA): Rationale, design and baseline data. MedRxiv. https://doi.org/0.1101/2021.04.09.21251911

Pallanti, S., & Salerno, L. (2020). Adult ADHD in Neurocognitive and Neurological Disorders. In The Burden of Adult ADHD in Comorbid Psychiatric and Neurological Disorders (pp. 287–305). Springer.

Paß, T., Aßfalg, M., Tolve, M., Blaess, S., Rothermel, M., Wiesner, R. J., & Ricke, K. M. (2020). The impact of mitochondrial dysfunction on dopaminergic neurons in the olfactory bulb and odor detection. Molecular Neurobiology, 57(9), 3646–3657. https://doi.org/10.1007/s12035-020-01947-w

Perlikowska, R. (2021). Whether short peptides are good candidates for future neuroprotective therapeutics? Peptides, 170528. https://doi.org/10.1016/j.peptides.2021.170528

Pinson, M. R., Chung, D. D., Adams, A. M., Scopice, C., Payne, E. A., Sivakumar, M., & Miranda, R. C. (2021). Extracellular Vesicles in Premature Aging and Diseases in Adulthood Due to Developmental Exposures. Aging and Disease, 0. https://doi.org/10.14336/AD.2021.0322

Ren, C., Wang, F., Guan, L.-N., Cheng, X.-Y., Zhang, C.-Y., Geng, D.-Q., & Liu, C.-F. (2019). A compendious summary of Parkinson's disease patient-derived iPSCs in the first decade. Annals of Translational Medicine, 7(22). https://doi.org/10.21037/atm.2019.11.16

Riessland, M., Kolisnyk, B., Kim, T. W., Cheng, J., Ni, J., Pearson, J. A., Park, E. J., Dam, K., Acehan, D., & Ramos-Espiritu, L. S. (2019). Loss of SATB1 induces p21-dependent cellular senescence in post-mitotic dopaminergic neurons. Cell Stem Cell, 25(4), 514–530. https://doi.org/10.1016/j.stem.2019.08.013

Ryan, B. J., Bengoa-Vergniory, N., Williamson, M., Kirkiz, E., Roberts, R., Corda, G., Sloan, M., Saqlain, S., Cherubini, M., & Poppinga, J. (2021). REST protects dopaminergic neurons from mitochondrial and α-synuclein oligomer pathology in an alpha synuclein overexpressing BAC-transgenic mouse model. Journal of Neuroscience, 41(16), 3731–3746. https://doi.org/10.1523/JNEUROSCI.1478-20.2021

Salado-Manzano, C., Perpiña, U., Straccia, M., Molina-Ruiz, F. J., Cozzi, E., Rosser, A. E., & Canals, J. M. (2020). Is the Immunological Response a Bottleneck for Cell Therapy in Neurodegenerative Diseases? Frontiers in Cellular Neuroscience, 14, 250. https://doi.org/10.3389/fncel.2020.00250

Salvatore, M. F., Soto, I., Alphonso, H., Cunningham, R., James, R., & Nejtek, V. A. (2021). Is there a Neurobiological Rationale for the Utility of the Iowa Gambling Task in Parkinson's Disease? Journal of Parkinson's Disease, Preprint, 1–15. https://doi.org/10.3233/JPD-202449

Sengupta, U., Puangmalai, N., Bhatt, N., Garcia, S., Zhao, Y., & Kayed, R. (2020). Polymorphic α-Synuclein Strains Modified by Dopamine and Docosahexaenoic Acid Interact Differentially with

Tau Protein. Molecular Neurobiology, 57(6), 2741–2765. https://doi.org/10.1007/s12035-020-01913-6

Serapide, M. F., L'Episcopo, F., Tirolo, C., Testa, N., Caniglia, S., Giachino, C., & Marchetti, B. (2020). Boosting Antioxidant Self-defenses by Grafting Astrocytes Rejuvenates the Aged Microenvironment and Mitigates Nigrostriatal Toxicity in Parkinsonian Brain via an Nrf2-Driven Wnt/β-Catenin Prosurvival Axis. Frontiers in Aging Neuroscience, 12, 24. https://doi.org/10.3389/fnagi.2020.00024

Siller, A., Hofer, N. T., Tomagra, G., Wiederspohn, N., Hess, S., Benkert, J., Gaifullina, A., Spaich, D., Duda, J., Pötschke, C., Vilusic, K., Fritz, E. M., Schneider, T., Kloppenburg, P., Liss, B., Carabelli, V., Carbone, E., Ortner, N. J., & Striessnig, J. (2021). Alternative splicing of auxiliary β2-subunits stabilizes Cav2.3 Ca2+ channel activity in continuously active midbrain dopamine neurons (p. 2021.02.10.430224). https://doi.org/10.1101/2021.02.10.430224

Simons, C., Benkert, J., Deuter, N., Poetschke, C., Pongs, O., Schneider, T., Duda, J., & Liss, B. (2019). NCS-1 deficiency affects mRNA levels of genes involved in regulation of ATP synthesis and mitochondrial stress in highly vulnerable Substantia nigra dopaminergic neurons. Frontiers in Molecular Neuroscience, 12, 252. https://doi.org/10.3389/fnmol.2019.00252

Singh, S. (2020). Noradrenergic pathways of locus coeruleus in Parkinson's and Alzheimer's pathology. International Journal of Neuroscience, 130(3), 251–261. https://doi.org/10.1080/00207454.2019.1667799

Snyder, J. S. (2019). Recalibrating the relevance of adult neurogenesis. Trends in Neurosciences, 42(3), 164–178. https://doi.org/10.1016/j.tins.2018.12.001

Stanojlovic, M., Pallais, J. P., & Kotz, C. M. (2021). Inhibition of Orexin/Hypocretin Neurons Ameliorates Elevated Physical Activity and Energy Expenditure in the A53T Mouse Model of Parkinson's Disease. International Journal of Molecular Sciences, 22(2), 795. https://doi.org/10.3390/ijms22020795

Subrahmanian, N., & LaVoie, M. J. (2021). Is there a special relationship between complex I activity and nigral neuronal loss in Parkinson's disease? A critical reappraisal. Brain Research, 147434. https://doi.org/10.1016/j.brainres.2021.147434

Surmeier, D. J. (2021). Beyond Just Connectivity-Neuronal Activity Drives α-Synuclein Pathology. Movement Disorders: Official Journal of the Movement Disorder Society, 36(7), 1487–1488. https://doi.org/10.1002/mds.28618

Swanson, O. K., Semaan, R., & Maffei, A. (2020). Reduced dopamine signaling impacts pyramidal neuron excitability in mouse motor cortex. BioRxiv. https://doi.org/10.1101/2020.02.12.946301

Tan, Y. (2020). The role of Fragile X Mental Retardation Protein in Parkinson's disease [PhD Thesis]. Technische Universität München.

Tan, Y., Sgobio, C., Arzberger, T., Machleid, F., Tang, Q., Findeis, E., Tost, J., Chakroun, T., Gao, P., & Höllerhage, M. (2020). Loss of fragile X mental retardation protein precedes Lewy pathology in Parkinson's disease. Acta Neuropathologica, 139(2), 319–345. https://doi.org/10.1007/s00401-019-02099-5

Tanaka, M., Toldi, J., & Vécsei, L. (2020). Exploring the etiological links behind neurodegenerative diseases: Inflammatory cytokines and bioactive kynurenines. International Journal of Molecular Sciences, 21(7), 2431. https://doi.org/10.3390/ijms21072431

Turconi, G., Alam, F., SenGupta, T., Pirnes-Karhu, S., Olfat, S., Schmidt, M. S., Mätlik, K., Montaño-Rodriguez, A., Heiskanen, V., & Piepponen, P. T. (2021). Nicotinamide riboside alleviates Parkinson's disease symptoms but downregulates dopamine metabolism upon lactacystin-induced proteostasis failure. BioRxiv. https://doi.org/10.1101/2021.03.12.435062

Tyer, I., & Hill, A. (2020). Novel Therapeutic Targets and Biomarkers for the Treatment of Progressive Supranuclear Palsy. https://doi.org/10.20944/preprints202006.0147.v1

Villar-Conde, S., Astillero-Lopez, V., Gonzalez-Rodriguez, M., Villanueva-Anguita, P., Saiz-Sanchez, D., Martinez-Marcos, A., Flores-Cuadrado, A., & Ubeda-Bañon, I. (2021). The Human Hippocampus in Parkinson's Disease: An Integrative Stereological and Proteomic Study. Journal of Parkinson's Disease, 11(3), 1345–1365. https://doi.org/10.3233/JPD-202465

von Linstow, C. U., DeLano-Taylor, M., Kordower, J. H., & Brundin, P. (2020). Does developmental variability in the number of midbrain dopamine neurons affect individual risk for sporadic Parkinson's disease? Journal of Parkinson's Disease, 10(2), 405–411. https://doi.org/10.3233/JPD-191877

Wong, Y. C., Luk, K., Purtell, K., Burke Nanni, S., Stoessl, A. J., Trudeau, L., Yue, Z., Krainc, D., Oertel, W., Obeso, J. A., & Volpicelli-Daley, L. A. (2019). Neuronal vulnerability in Parkinson disease: Should the focus be on axons and synaptic terminals? Movement Disorders, 34(10), 1406–1422. https://doi.org/10.1002/mds.27823

Yang, P., Knight, W. C., Li, H., Guo, Y., Perlmutter, J. S., Benzinger, T. L., Morris, J. C., & Xu, J. (2021). Dopamine D1+ D3 receptor density may correlate with parkinson disease clinical features. Annals of Clinical and Translational Neurology, 8(1), 224–237. https://doi.org/10.1002/acn3.51274

Yoshino, K., Inomoto, S., Iyama, A., & Sakoda, S. (2021). Dynamic sleep stage transition process analysis in patients with Parkinson's disease having sleep apnea syndrome. Informatics in Medicine Unlocked, 25, 100656. https://doi.org/10.1016/j.imu.2021.100656

Young, M. (2021). Targeting Pathological Protein Interactions in Drug Discovery [PhD Thesis]. University of Minnesota.

Zampese, E., & Surmeier, D. J. (2020). Calcium, Bioenergetics, and Parkinson's Disease. Cells, 9(9), 2045. https://doi.org/10.3390/cells9092045

Zhan, Y., Raza, M. U., Yuan, L., & Zhu, M.-Y. (2019). Critical Role of Oxidatively Damaged DNA in Selective Noradrenergic Vulnerability. Neuroscience, 422, 184–201. https://doi.org/10.1016/j.neuroscience.2019.09.036

13 Chapter 4 — Axonal domain structure and function as a key cell-autonomous characteristic of selective vulnerability in Parkinson's disease — a murine study of primary cultured neurons

Overview

In this study we aimed to test the hypothesis that neuronal populations vulnerable in PD grow larger and more complex axonal arborizations than non-vulnerable populations. Furthermore, we hypothesized that these PD-vulnerable neurons would, at basal levels, produce higher levels of oxidative stress, and in turn, would be more vulnerable to cell stress induced by hydrogen peroxide — a potent oxidative stressor. We recognize that, given our previous discussions on the identity of "vulnerable" vs "non-vulnerable", this binarization is perhaps somewhat questionable. However, we believe that by comparing the vulnerability of different neuronal populations, in the context of assays relevant to the pathophysiology of PD, we are able to reveal insights relevant to cell-autonomous factors such as the morphology and physiology of the axonal domain, that may be important for neuronal vulnerability in the context of PD.

Contributions

Samuel Burke: conception, data collection, analysis, writing of manuscript.

Louis-Eric Trudeau: conception, providing resources, analysis, supervision, writing of manuscript.

In revision eNeuro.

Axonal domain structure and function as a key cell-autonomous characteristic of selective vulnerability in Parkinson's disease — a murine study of primary cultured neurons

Samuel Burke [1,2,3] and Louis-Eric Trudeau[1,2,3]

1. Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal
2. Department of Neurosciences, Faculty of Medicine, Université de Montréal
3. Neural Signaling and Circuitry Research Group (SNC), Montréal, QC, Canada

13.01 Abstract

The motor symptoms of Parkinson's disease (PD) are linked to the degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNc).Several populations of neurons are purported to degenerate in PD. . One current hypothesis suggests that vulnerable neurons in PD share common morphological characteristics including projecting to voluminous territories and having extremely long and branched axonal domains with large numbers of neurotransmitter release sites. In this study, we used a mouse *in vitro* culture system to compare the axonal domain of neuronal populations suspected to be vulnerable in PD to that of neuronal population considered at a lesser risk. In the first group, we included DA neurons of the SNc, noradrenergic neurons of the locus coeruleus (LC), serotonin neurons of the raphe nuclei (R), and cholinergic (ChAT+) neurons of the dorsal motor nucleus of the vagus (DMV). In the second group, we included DA neurons of the VTA, cholinergic neurons of the hypoglossal nucleus (XII), and cholinergic interneurons of the dorsal striatum (STR). Validating their differential vulnerability, we find that, when compared to PD-resilient neurons, a larger proportion of PD-vulnerable neurons die in the presence of hydrogen peroxide, despite similar levels of reactive oxygen species production in mitochondria. We also find that they are endowed with larger axonal domains, that are more complex, have more axonal varicosities and with a higher proportion of varicosities that are positive for synaptotagmin 1. Globally, these findings support the hypothesis that axonal domain structure and function is a key cell-autonomous characteristic of vulnerability.

13.02 Significance statement

Parkinson's disease (PD) causes the specific degeneration of a small number of neuroanatomically and neurochemically defined neuronal populations. Currently, our hypotheses suggest that these neurons are vulnerable due to their specific physiology and morphology. In this study — using mouse primary neurons — we demonstrate that, when compared to comparable neuronal populations that are not vulnerable in PD, PD-vulnerable neuron are more vulnerable to cell stress induced by hydrogen peroxide, and that the overt length and complexity of their axonal arborizations is larger. Furthermore, the striking difference

between the comparable projecting neurons is the number of Synaptotagmin1 positive axonal varicosities: a proxy for their functional status.

13.03 Introduction

There are no disease modifying treatments available for people living with Parkinson's Disease (PD). This is clearly related to our limited understanding of why varying PD-related risk factors converge in causing selective dysfunction and degeneration of several neurochemically and anatomically restricted neuronal populations. A better understanding of the origin of selective neuronal vulnerability in PD is therefore critical.

Canonically, PD pathology is described by the presence of Lewy bodies in the brain (for a historical review see (Goedert et al., 2013)) and by the loss of neuromelanin-containing dopamine (DA) neurons in the substantia nigra pars compacta (SNc). However, the relationship between Lewy pathology and cell loss in PD is unclear and has not unequivocally revealed why certain neurons degenerate, while others do not (Espay & Lang, 2018; Surmeier et al., 2017b). Importantly, the pathology and degeneration in PD is not limited to dopamine (DA) neurons of the SNc, but rather appears to occur in several nuclei, including the locus coeruleus (Huynh et al., 2021) and pedunculopontine nucleus (Hirsch et al., 1987; Tubert et al., 2019), although more systematic stereological quantifications are clearly required to confirm the nuclei showing frank cell loss and not only Lewy pathology, as well as the sequence of this cell loss (Giguère et al., 2018). A better understanding of what determines the vulnerability of neurons that are affected in PD is essential for progress (Surmeier et al., 2017a).

An increasing amount of work supports the hypothesis that the neurons that are most vulnerable in PD share distinguishing morphological and physiological characteristics – cell-autonomous factors – that render them selectively vulnerable. Among these, two have been increasingly gaining experimental support. The first is autonomous pacemaking, with broad spikes, and high intracellular calcium oscillations (Surmeier et al., 2017a). The second is being endowed with a very long, and highly branched axonal domain, with orders of magnitude more neurotransmitter-release sites than most other neuron types (Pacelli et al., 2015; Parent & Parent, 2006; Pissadaki & Bolam, 2013; Wong et al., 2019). It is thought that these features converge in leading to elevated bioenergetic demands and an associated high level of chronic oxidative stress (Pissadaki & Bolam, 2013), making the neurons less resilient to mitochondrial dysfunction, proteostatic burden (Lehtonen et al., 2019) or dysfunction in essential cellular systems such as the endo-lysosomal system (Vidyadhara et al., 2019).

However, two important foundations underlying this working hypothesis require additional investigation. One, is the purported identity of PD-vulnerable neurons, which is still unclear because comparative stereological counting of multiple nuclei in post-mortem brains from PD subjects has not been achieved (Giguère et al., 2018; Lunt et al., 2021). And two, work comparing cell-autonomous factors in PD-vulnerable neuronal populations has been mainly limited to a comparison of SNc and VTA neurons (albeit some exceptions (Goldberg et al., 2012; Sanchez–Padilla et al., 2014)).

Previous work has shown that cell-autonomous differential vulnerability of SNc and VTA DA neurons is maintained *in vitro*, including a striking correlation between bioenergetic demands, vulnerability to PD-relevant cell stress, and axonal arbor size (Pacelli et al., 2015). In this present study we use a similar *in vitro* system, where we examine the characteristics and vulnerability of several PD-relevant neuronal populations, with the objective to evaluate the hypothesis that the outright size, complexity, and extensive number of neurotransmitter release sites is linked to vulnerability. We compared neurons from regions suspected to be vulnerable in PD (DA neurons of the SNc, noradrenergic neurons of the locus coeruleus (LC), serotonin neurons of the raphe nuclei (R), and cholinergic neurons of the dorsal motor nucleus of the vagus (DMV) to neurons from regions classically hypothesized as more resilient in PD (DA neurons of the VTA (although their vulnerability in PD is controversial (Alberico et al., 2015)), cholinergic neurons of the hypoglossal nucleus (XII), and cholinergic interneurons of the dorsal striatum (STR)).

We find that globally, neurons previously suspected to be vulnerable in PD are less resilient in response to cell stress induced by hydrogen peroxide, except for cholinergic neurons of the DMV. In keeping with the hypothesis of a link between axonal domain complexity and vulnerability, we also find that these PD-vulnerable neurons, on average, have longer and more complex axonal domains, with a much higher proportion of varicosities containing the Ca^{2+} sensor synaptotagmin 1 (Syt1) and that are thus likely active. Together these findings support the notion of a link between axonal complexity and basal vulnerability of neurons in PD.

13.04 Materials and Methods

Animals Procedures with animals and their care were conducted in accordance with the Guide to care and use of Experimental Animals of the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). Experimental protocols were approved by the animal ethics committees of the Université de Montréal. Housing was at a constant temperature of 21°C and humidity of 60%, under a fixed 12-hour light / dark cycle, with food and water available ad libitum.

Transgenic animals used All mice were maintained as heterozygotes.

TH-GFP The TH-green fluorescent protein (GFP) transgenic mouse line TH-EGFP/21–31, which carries the enhanced GFP gene under control of the TH promoter (Matsushita et al., 2002), was maintained on a C57/BL6 background.

DAT-Ai9 Dat-Ires-Cre animals (catalog #006660, The Jackson Laboratory; (Bäckman et al., 2006)) were crossed with Ai9/tdTomato mice (catalog #007905, The Jackson Laboratory; (Madisen et al., 2010)), allowing conditional expression of the red fluorescent protein tdTomato in DA neurons.

ChAT-Ai9 ChAT-IRES-Cre (catalog #006410, The Jackson Laboratory; (Rossi et al., 2011)) were crossed with Ai9/tdTomato mice (catalog #007905, The Jackson Laboratory; (Madisen et al., 2010)) allowing conditional expression of the red fluorescent protein tdTomato in cholinergic neurons.

SERT-Ai9 Slc6a4-Cre (MMRRC Stock #031028-UCD; (Gong et al., 2007)) were crossed with Ai9 (catalog #007905, The Jackson Laboratory; (Madisen et al., 2010)) mice, allowing conditional expression of the red fluorescent protein tdTomato in serotonin neurons.

Primary cell culture Primary neuron cultures were prepared from dissections of male and female P0 mice as described previously with slight modification (**Figure 21A**) (Fasano et al., 2008). **Figure 21B** shows the anatomical location of target structures used in this work. For all experiments, cells were cultured for 10 days, prior to either fixation, or live cell imaging. All experiments were performed on at least 3 (3-6) independent cultures. For analysis of varicosities, cultures were prepared as previously described, and seeded onto 15mm cell adhesion-treated glass coverslips (65µl of 100,000 cells / ml) (Fasano et al., 2008). For hydrogen peroxide assays concentrations of 0, 100, 150, and 200µM were used, as these have been found to generate consistent decreases in neuronal survival across neurons types (Pacelli et al., 2015).

96 well plates For cell stress assays and neurite tracing, primary cultures were prepared as described above, but seeded into 96 well plates (µ-Plate 96 Well Black, ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized; Cat.No. 89626). Since dissections of tissue surrounding targeted neurochemically and anatomically defined nuclei can vary, we pooled cells from, 3-5 post-natal day 0-2 pups, per multi well plate. This enables a reduction in, for example, total DA SNc neurons variability — culture to culture — even where total number of cells (neurons and glia) seeded varied. This resulted in ~ 5k to 10k cells, in total, being seeded per well. For cell stress assays, hydrogen peroxide (30% (W/W) solution, Sigma H-4381) was added at 0, 100, 150, and 200µM, at 10 days *in vitro* (DIV), and cells were fixed at 11 DIV.

Live cell imaging For live cell imaging, cultures were prepared as above, but seeded (200µl of 100,000 cells / ml) into 35mm imaging compatible glass-bottomed petris (µ-Dish 35 mm, high ibiTreat: 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized; Cat.No. 81156).

Immunocytochemistry Cultures were fixed with 4% paraformaldehyde (PFA; in PBS, pH-7.4) at 10 DIV, permeabilized with 0,1% triton X-100 during 20-min, and nonspecific binding sites were blocked with 10% bovine serum albumin for 10 min. Primary antibodies were incubated overnight at room temperature: Anti-Tyrosine Hydroxylase (1:2000, AB152, Cedarlane), Anti-MAP-2 (1:2000, MAB3418, Millipore Sigma), Anti-RFP (1:1000, 600-401-379, Cedarlane), Anti-Syt1 (1:200, 105 102, Synaptic Systems). These were subsequently detected using Alexa Fluor-488-conjugated, Alexa Fluor-546-conjugated, Alexa Fluor-568-conjugated, or Alexa Fluor-647-conjugated secondary antibodies (incubated at room temperature at 1:1000 for 1 hour, Invitrogen).

Mitochondrial targeting of the ROS sensor, roGFP For all primary neuron cultures in a cre-backround, 1µl of AAV9-CMV-DIO-MTS-roGFP-WPRE-bGHpA (~2-3 × 10^{13} vector genome ml−1 titers) was added to cultures at 1 DIV and for cultures targeting MTS-roGFP to LC neurons, in C57 primary neurons, 1µl AAV9-TH-MTSroGFP (~2-3 × 10^{13} vector genome ml−1 titers). These tools (Sanchez–Padilla et al., 2014) were kind gifts from the laboratory of D James Surmeier (Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA).

Imaging & data analysis

Image acquisition Confocal imaging was carried out on an Olympus Fluoview FV1000 point-scanning confocal microscope (Olympus, Tokyo, Japan). Images were scanned sequentially to prevent non-specific bleed-through signal using 488, 546, and 647-nm laser excitation and a 60X (NA 1:42) oil immersion objective. All other imaging was acquired on a Nikon Eclipse Ti2-E inverted microscope, using either a CFI Plan Apo Lambda 20X objective (for cell counting and neurite tracing), or a CFI Plan Apo Lambda 60X oil immersion objective (for live cell imaging).

Image processing and analysis, data analysis, and statistics Exploratory image visualization and analysis was done using Napari (Nicholas Sofroniew et al., 2021). Subsequently, unmodified images were all processed using ImageJ (Schneider et al., 2012), and custom analysis scripts written in ImageJ Macro language [https://github.com/samuelorion/burke-trudeau-2022]).

Raw data availability Due to the considerable volume of imaging data (> 5 TBs) generated in the present study, sharing of the primary data on an open data-storage solutions was not possible. However, these data are available upon request.

Image analysis

Counts and neurite tracing To conduct unbiased and high-throughput quantifications of neuron numbers, we developed our own algorithm to count projecting neurons [Chapter 5]. Briefly, images were processed for segmentation to identity cell bodies. Segmentations were then compared to raw images and validated for accuracy, where we achieved ~90% accuracy across neuron types [Chapter 5] with minimal computational requirements. A slightly modified version [https://github.com/samuelorion/manuscript-burke-trudeau] of the above system was adapted to achieve similar performance in tracing neurites, for quantification (Figure 5 and 6).

ROS Imaging experiments were performed at room temperature (20–22 °C) because previous studies showed that probe oxidation was nearly complete at physiological temperatures (Sanchez–Padilla et al., 2014). Regions of interest (ROIs) were generated using an automatic segmentation approach [https://github.com/samuelorion/manuscript-burke-trudeau], where GFP positive puncta were segmented and used to measure fluorescence intensity. Recordings where a drifting baseline of more than 10% was detected – due to photobleaching or photo-oxidation of roGFP – were not included. The maximum and minimum fluorescence of mito-roGFP was determined according to a previously described procedure (Guzman et al., 2010), by application of 2 mM dithiothreitol (DTT) (to reduce the mitochondria fully), and then 100 µM aldrithiol (Ald) (to oxidize the mitochondria fully). ROIs that did not responds to DTT and Ald were not included. The relative oxidation level was then calculated as $1 - [(F - FAld)/(FDTT - FAld)]$, where *F* represents, fluorescence intensity, at baseline, FDTT, F in the presence of DTT, and FAld, F in the presence of ALD. Image alignment was done using the ImageJ plugin StackReg (Thévenaz et al., 1998).

Varicosities Confocal images were processed [https://github.com/samuelorion/burke-trudeau-2022] and varicosities were segmented. Varicosities were defined as enlargements along thin neurites with a measured width between 0.2–1µm, and a length of 0.3–0.5µm. Segmentations were then mapped onto images of Syt1, where fluorescence intensity was quantified. To determine the status of Syt1 positivity (Figure 7 A; Figure 8 B), we estimated the intensity of syt1 signal in segmentations that were excluded and used this value as a cut off for Syt1 positive status.

Statistics Given previous work in our group quantifying neuron numbers on coverslips, we conducted an a priori power analysis to detect an effect size of 25%, with a power of 80%, and an alpha = 0.05, concluding our requirement for n to be 22 for survival assays. We therefore aimed for this. However, for some wells, images were excluded due to contamination. For all other experiments we aimed to have an n of at least 12. For statistical analyses and data visualization we used R(R Core Team, 2017), and the subsequent packages for all statistical analyses, and data visualisation (*Data Analysis Using Bootstrap-Coupled Estimation [R Package Dabestr Version 0.3.0]*, 2020; *RStudio | Open Source & Professional Software for Data Science Teams*, n.d.; *Simple Fisheries Stock Assessment Methods [r Package Fsa Version 0. 8. 32]*, 2021; Müller,

2020; Pedersen, 2021; Slowikowski, 2021; Wickham, 2021, 2021; Wilke, 2021). For each statistical analysis, we evaluated whether the data were parametric or non-parametric, and subsequently conducted appropriate analyses, including appropriate post-hoc multiple comparisons: described in the available supplementary .Rmd file (https://github.com/samuelorion/burke-trudeau-2022). In figures, we include an asterisk to indicate that, for an alpha = 0.05, there is a significant difference compared to the value for the SNc (our reference neuronal population). All other post-hoc comparisons can be found in supplementary .Rmd file. All figures include box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles), and the whiskers extend to the largest and smallest value (no further than 1.5 times the interquartile range). Furthermore, where appropriate, we include individual data points. Following null hypothesis testing, we also performed estimation based on confidence intervals using the R package *dabestr* (Ho et al., 2019). For all experiments, we conducted shared control Cumming plots, compared to the SNc. These Cumming plots have been placed below plots, below null hypothesis testing. Furthermore, analyses were conducted comparing "PD-vulnerable" to "PD-resilient" neurons, where Gardner-Altman two-group estimation plots are plotted next to main box and whisker plots.

13.05 Results

We used a mouse primary culture system in which neurons from multiple brain PD-relevant regions were grown on supporting astrocytes (Figure 21). These were subsequently neurochemically defined, identifying them either by immunocytochemistry for their neurochemical identity, or the presence of the fluorescent reporter protein TdTomato, expressed in a cre-dependent manner. For this study we chose four nuclei that are considered vulnerable in PD, including DA neurons of the SNc, noradrenergic neurons of the LC, serotonin neurons of the raphe nuclei, and cholinergic (ChAT+) neurons of the dorsal motor nucleus of the vagus (DMV). We compared these to neuronal populations considered more resilient in PD, including DA neurons of the VTA, cholinergic neurons of the hypoglossal nucleus (XII), and cholinergic interneurons of the dorsal striatum (STR).

Figure 1

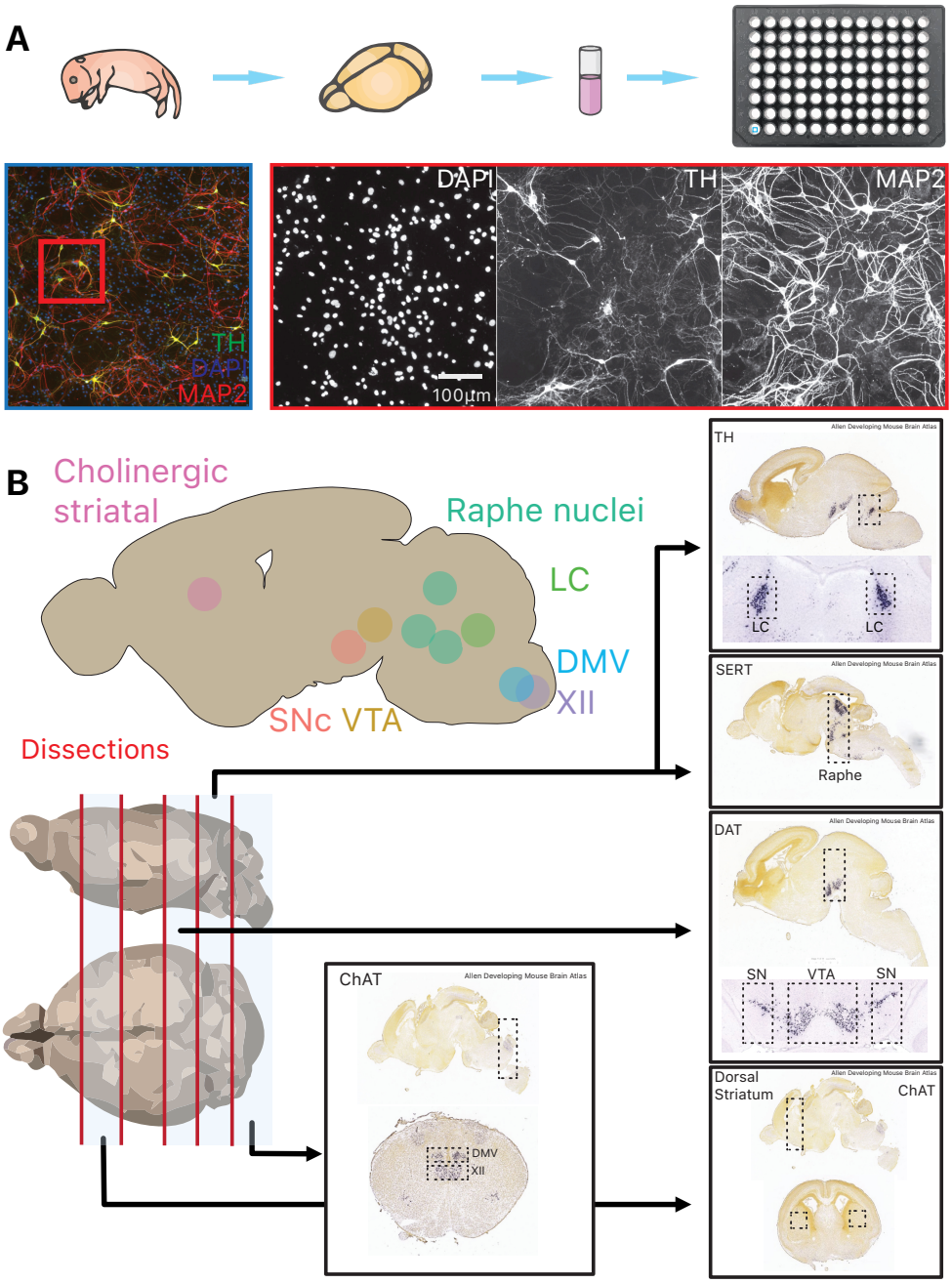


Figure 21 Overview of experimental paradigm, neuroanatomical, and neurochemical identify of target structures cultured

A) Post-natal day 0-2 mice pups had their brains dissected, and target structures isolated, and cultured in 96-well plates for 10 DIV. **B**) Overview of the eight target structures and subsequent dissection strategy in 4 transgenic mice lines (TH-GFP, DAT-Ai9, ChAT-Ai9, and SERT-Ai9).

Additional images from the Allen Developing Mouse Brain Atlas (ISH Data :: Allen Brain Atlas: Developing Mouse Brain, 2008; Lein et al., 2007).

PD-vulnerable neurons are less resilient to cell stress induced by hydrogen peroxide

Previous work has shown that in line with *in vivo* observations, DA neurons of the SNc *in vitro* are more vulnerable than DA neurons of the VTA to cell stress induced by several PD-relevant stressors (Lieberman et al., 2017; Pacelli et al., 2015). Here we extended such a comparative analysis of neuronal vulnerability in a much larger set of neuronal populations with distinct neurochemical identities. Considering the well-established contribution of oxidative stress to PD pathophysiology, we compared the survival of neurons exposed to three concentrations of hydrogen peroxide (100, 150 and 200 μM), a cell stressor that is expected to act on all types of neurons (**Figure 22**). A quantification of neurochemically-defined neurons in these cultures revealed that, considered as a group, neurons from "PD-vulnerable" nuclei (SNc, LC, Raphe, DMV) were more sensitive to hydrogen peroxide compared to neurons from "PD-resilient" nuclei (VTA, XII, STR (**Figure 22C** and **Figure 2-1**).

A closer examination of the relative vulnerability of each neuronal population across the increasing doses of hydrogen peroxide (**Figure 23**) revealed significant effects of hydrogen peroxide at doses of 100, 150, and 200μM (**Figure 3-1**). Notably, at 150 μM hydrogen peroxide, cholinergic neurons of the DMV, hypoglossal nucleus and striatum showed less neuronal loss compared to SNc DA neurons, ChAT+ neurons of the DMV (unpaired mean difference of DMV (n = 16) minus SNc (n = 20) 0.197 [95CI 0.0662; 0.34]), ChAT+ neurons of the XII (Unpaired mean difference of XII (n = 23) minus SNc (n = 20) 0.152 [95CI -0.0443; 0.388]), and ChAT+ neurons of the STR (Unpaired mean difference of STR (n = 12) minus SNc (n = 20) 0.581 [95CI 0.35; 0.831]. In comparison, LC and Raphe neurons showed cell loss comparable to SNc DA neurons. VTA DA neurons also showed a tendency for reduced cell loss compared to SNc DA neurons, although this did not reach significance (**Figure 23**). This differential vulnerability to hydrogen peroxide persisted at 200μM, with VTA DA neurons and all cholinergic groups showing reduced vulnerability (**Figure 23**). Except for the DMV — hypothesized to be vulnerable in PD — our findings are in keeping with the hypothesis that in addition to DA neurons of the SNc, noradrenergic LC neurons and serotonergic Raphe neurons show elevated intrinsic vulnerability.

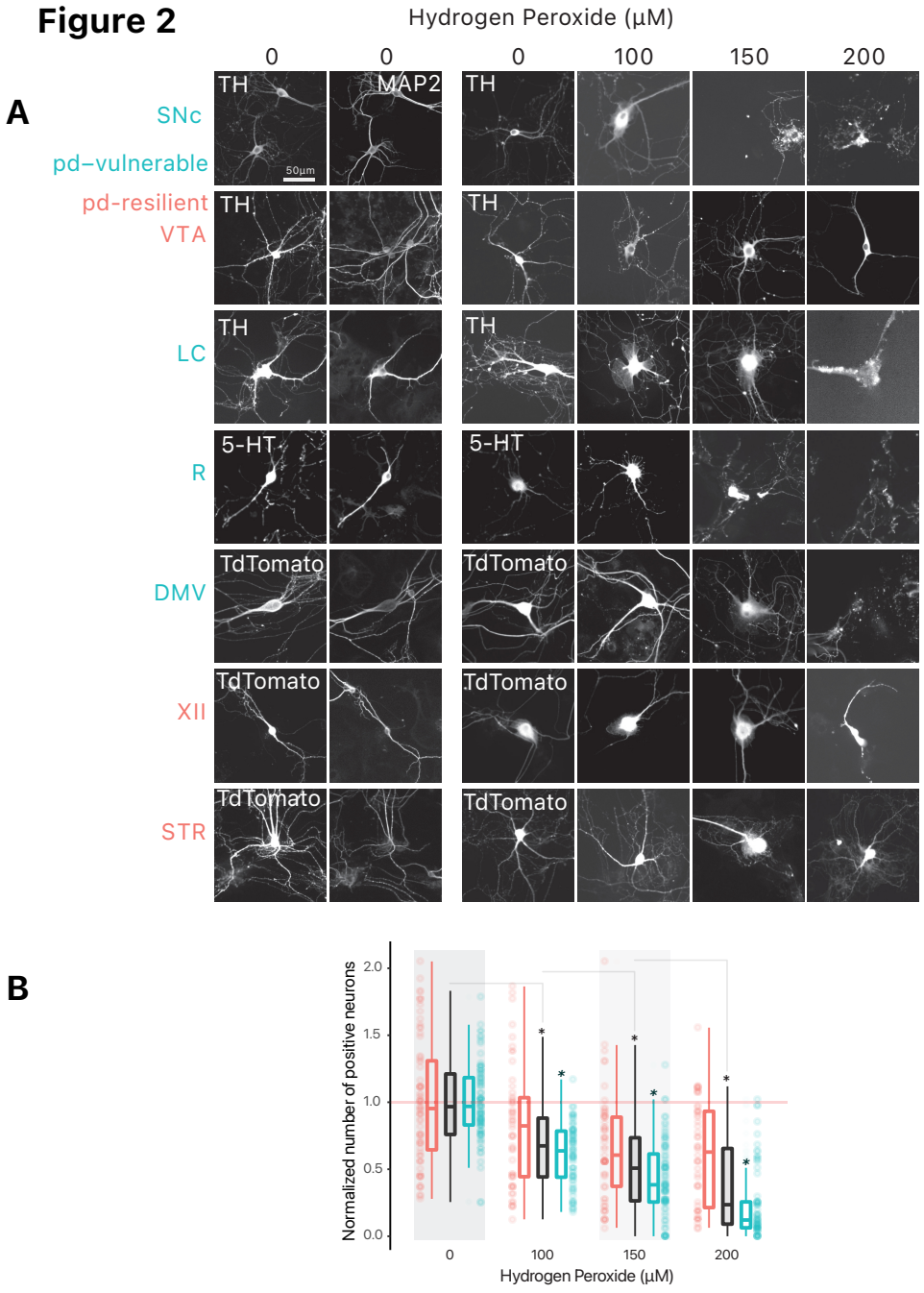


Figure 22 PD-vulnerable neurons are more vulnerable to hydrogen peroxide than pd-resilient neurons

Neurons were treated with hydrogen peroxide at 10 DIV, and fixed at 11 DIV. **A**) Example photomicrographs of all positive-identified neuron types across the PD-vulnerable, and PD-resilient target structures. **B**) Normalized number of positive-neurons across hydrogen peroxide concentrations. Box and whiskers plots, in the style of Tukey, where the median value is indicated, and the lower and upper hinges correspond to the first and third quartiles. * = one-way ANOVA, Tukey's HSD, $p<0.05$; *= *pairwise t-test, pd-vulnerable vs pd-resilient, $p<0.05$.*

Figure 3

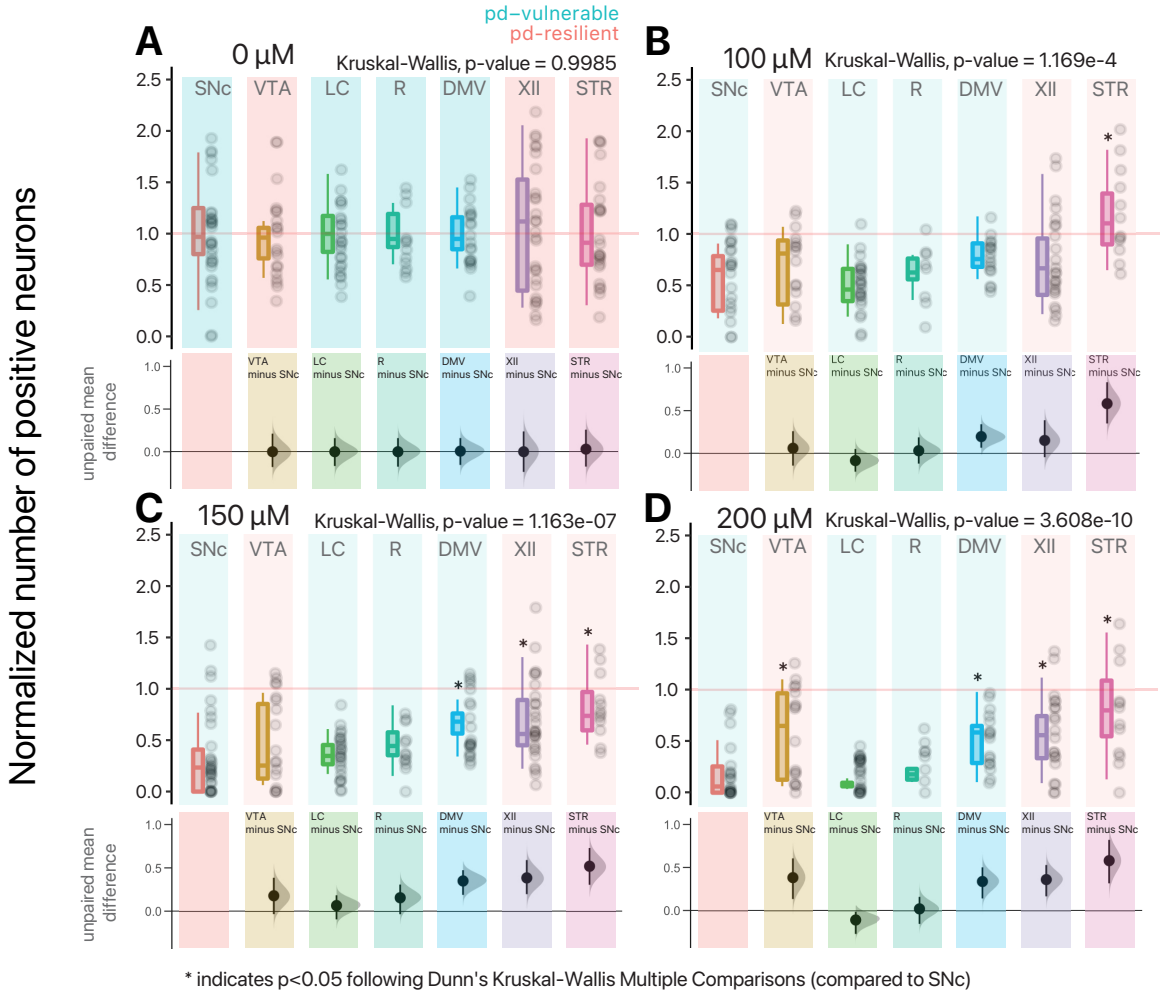


Figure 23 Differential vulnerability of neurons to hydrogen peroxide

Normalized number of positive-neurons, for each concentration of hydrogen peroxide, and neuronal population. Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = p < 0.05. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated.

ROS production is elevated in the somatodendritic domain in PD-vulnerable neurons.

The origin of the elevated vulnerability of SNc and other vulnerable neurons in PD has been previously hypothesized to result at least in part from their particularly elevated bioenergetic demands, leading to higher rates of mitochondrial oxidative phosphorylation and chronically elevated levels of

oxidative stress (Bolam & Pissadaki, 2012; Pacelli et al., 2015). We therefore examined basal mitochondrially-derived ROS production using the ROS sensitive GFP probe, mito-roGFP. The construct was expressed in neurons using a Cre recombinase-dependant AAV vector or a TH promotor (for LC neurons) (**Figure 24A**). The selective expression of mito-roGFP was validated by confirming that expression was limited to tdTomato-expressing neurons or to neurons positive for TH (for LC neurons, see (**Figure 1-2**)). Experiments were conducted by live time-lapse imaging of baseline mito-roGFP fluorescence, followed by a determination of the dynamic range of the reporter by measuring the fluorescence increase induced by the reducing agent DTT and the fluorescence decrease induced by the oxidant molecule aldrithiol (ALD) (**Figure 24B**). The signal was quantified both in the neurons' somatodendritic domain and in the neurons' axonal fields (**Figure 24A**). Among the neurons examined, a broad range of basal oxidant levels were identified, with some mitochondria within neurons showing low basal oxidation and others showing high basal oxidation (**Figure 24C**). These experiments revealed that, contrary to expectation, only ChAT+ DMV neurons had significantly reduced relative oxidation, when compared to the DA SNc neurons (**Figure 24C, Figure 4-1**). Furthermore, intermitochondrial distances within the axonal domain were comparable across neuron types, with a mean of ~ 13µm (SD of 5.4) (**Figure 24D, Figure 4-2**).

Figure 4

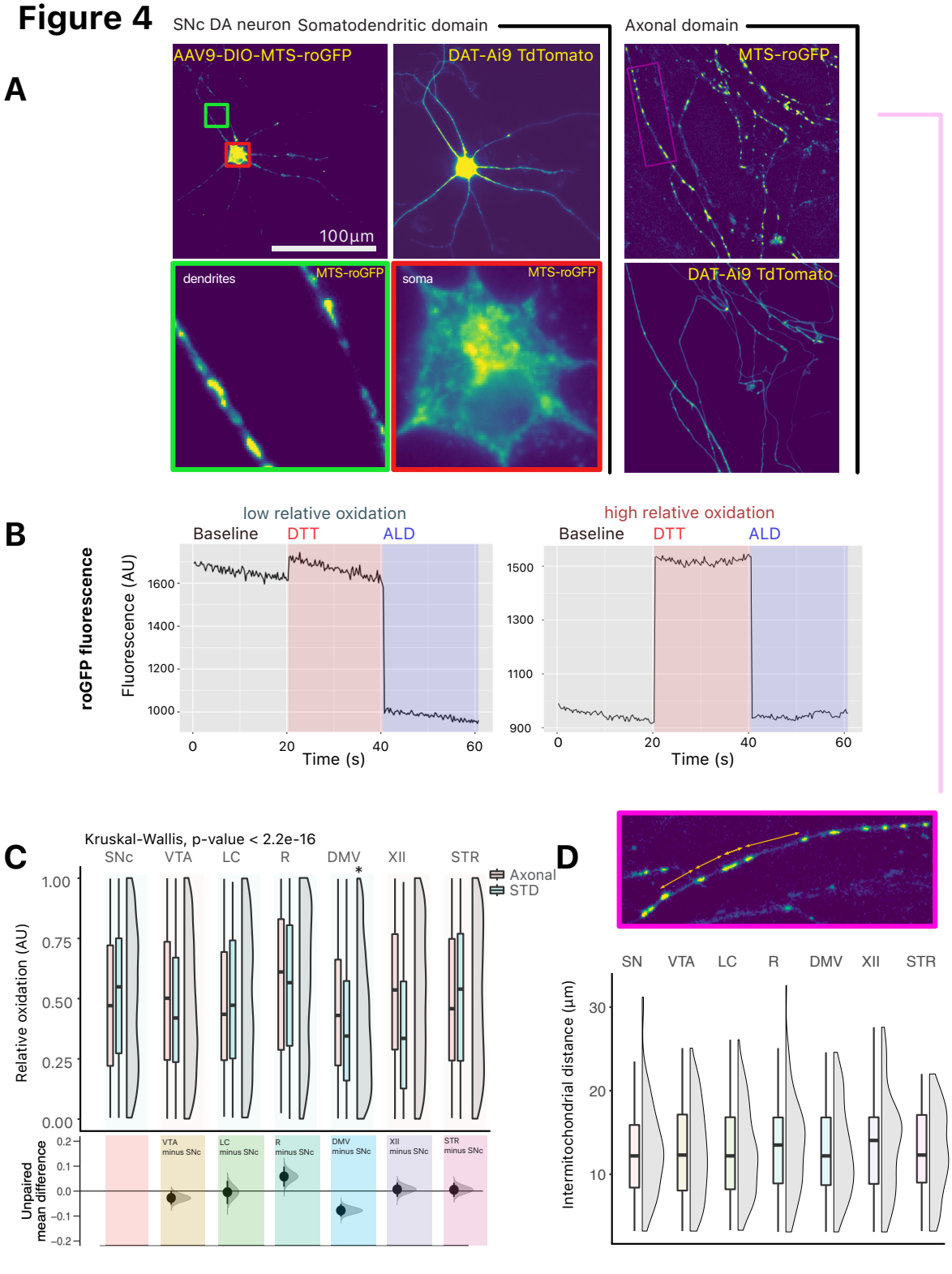


Figure 24 No overt difference in mitochondrial ROS production is observed between neurons

A) The redux sensitive GFP, roGFP, was expressed in a cre-dependent manner in target neurons. Photomicrographs of roGFP in a TdTomato-positive SNc DA neuron, in the somatodendritic compartment and in the axonal compartment. **B**) Example traces of GFP fluorescence arbitrary units (AU) in responsive ROIs, in an SNc DA neuron. Two traces are shown: for a GFP-positive puncta that showing low relative oxidation status, and for a GFP-positive puncta showing high relative oxidation status. **C**) Quantification of the relative oxidative state of mitochondria across neuron types. Box and whiskers plots, in the style of Tukey, where the median value is indicated, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p < 0.05$. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **D)** Quantification of intermitochondrial distance in the axonal domain (measured from the centre of each GFP positive puncta). Box and whiskers plots, in the style of Tukey, where the median value is indicated, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis $p>0.05$. Density plots show distribution of individual measurements.

PD-vulnerable neurons have large axonal domains, that are more complex than PD-resilient neurons

Previous work has shown that murine DA SNc neurons have larger and more complex axonal arbors compared to VTA DA neurons, both *in vitro* (Pacelli et al., 2015), and *in vivo* (Giguère et al., 2019). This observation is in line with the hypothesis that the total length and complexity of the axonal domain is a cell-autonomous feature that contributes to rendering these cells most vulnerable because of its associated bioenergetic burden. Here, given our working hypothesis, we examined the morphology of the axonal domain of each neuronal population. Given the number of neuron sub-types evaluated in this study, we developed simple and robust methods to quantify the axonal domain of these projecting neurons — all quantifications normalized to the number of neurons within each well (**Figure 25A, 25B, 26A**). Given that the majority of neurites detected in these neurons were MAP-2 negative, and thus not dendrites, we considered the neurons' somatodendritic domain as being negligeable in size compared to the axonal domain (**Figure 25A**) and therefore quantified neurite length as a proxy for axonal length. We find that there is a significant difference in mean total neurite length when comparing PD-vulnerable to resilient neurons (**Figure 26B**, **Figure 6-1**). Surprisingly, only VTA DA neurons were significantly different compared to SNc DA neurons in terms of mean neurite length per neuron (Unpaired mean difference of VTA (n = 22) minus SNc (n = 21) -9450 [95CI -16900; -5080]). However, using data analysis with bootstrap estimation suggests that both hypoglossal and striatal cholinergic neurons also have, on average, shorter axonal arborizations (Unpaired mean difference of XII (n = 19) minus SNc (n = 21) -6940 [95CI -15300; -1850], Unpaired mean difference of STR (n = 14) minus SNc (n = 21) -5660 [95CI -13700; -190]. We also estimated axonal arbor complexity by measuring the total number of segmentations and average length of segmentations (**Figure 26C, 26D**). PD-vulnerable neurons had far more segmentations per neuron that PD-resilient neurons. We find that, compared to SNc DA neurons, VTA DA neurons and hypoglossal neurons have substantially fewer segmentations per neuron (**Figure 26C**, **Figure 6-1**). We finally estimated

the average length per branch of axon, where no overt difference were observed between PD-vulnerable and resilient neurons. However, VTA DA neurons, LC noradrenergic neurons, DMV cholinergic neurons and hypoglossal cholinergic neurons had longer segmented neurite length compared to SNc DA neurons (**Figure 26D**, **Figure 6-1**) (Unpaired mean difference of VTA (n = 22) minus SNc (n = 21) 64.6 [95CI 31.4; 99.1], Unpaired mean difference of DMV (n = 30) minus SNc (n = 21) 77.3 [95CI 50.9; 104]). Together these results suggest that even though inconsistencies were observed, globally, mean total neurite length is smaller in neuronal populations suspected to be more resilient in PD and the total number of neurites were also smaller in these neurons.

Figure 5

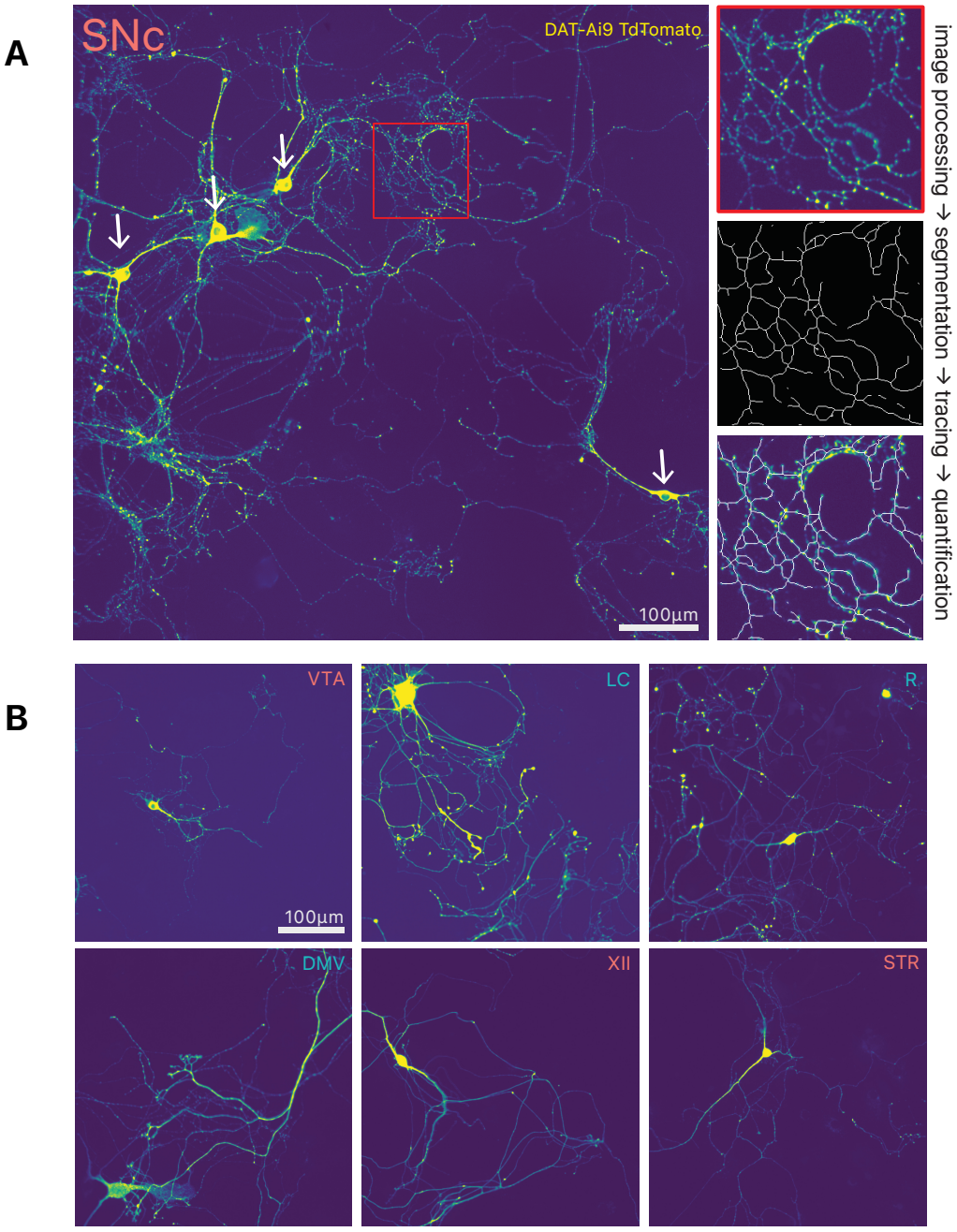


Figure 25 Overview of neurite tracing for quantification

A) Photomicrograph of SNc DA neurons, and overview of neurite quantification method. **B**) Photomicrographs fields of neurons, with their neurochemical marker immunocytochemistry.

Figure 6

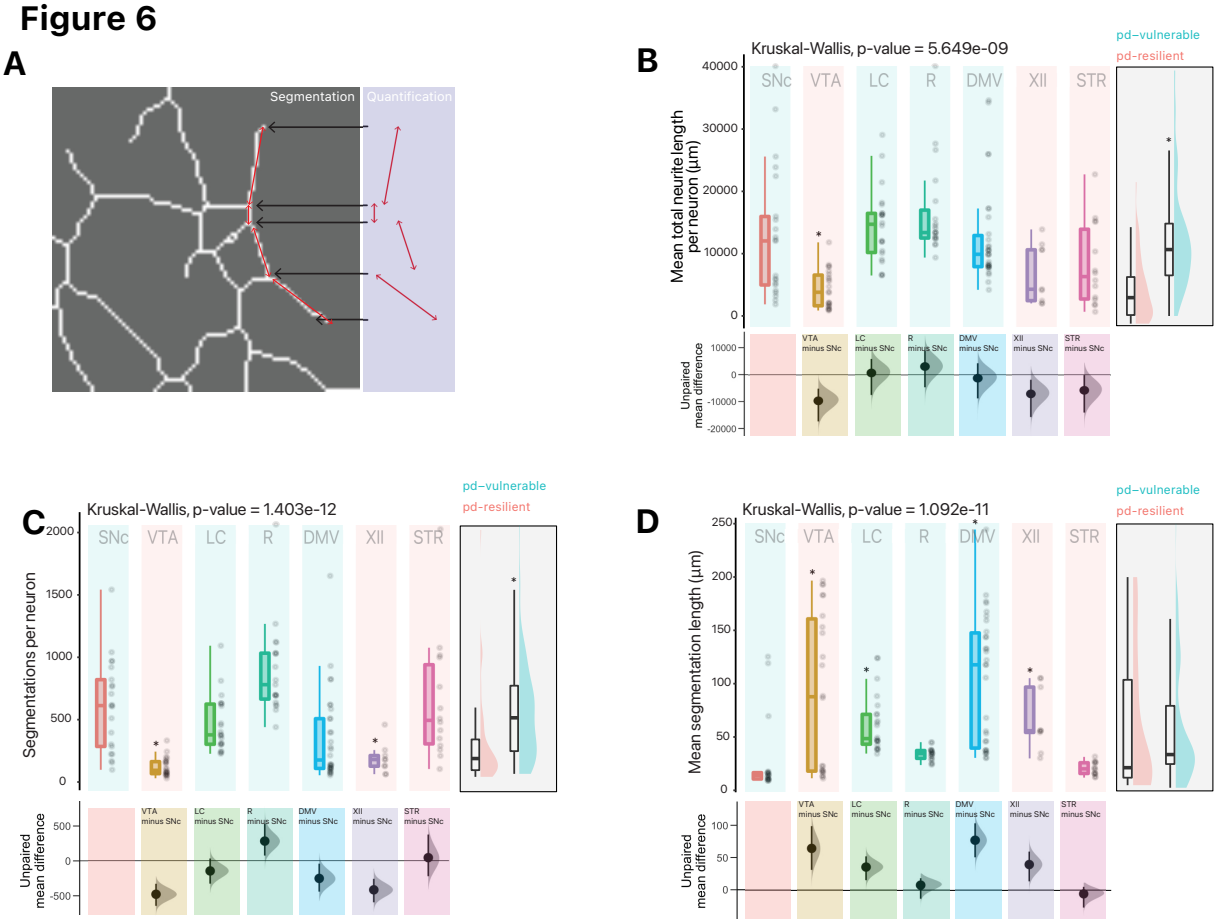


Figure 26 PD-vulnerable neurons have large axonal domains, that are globally more complex than PD-resilient neurons

A) Overview of quantification method for neurite segmentations. **B**) Quantification of mean neurite length (total neurite length, per well, divided by number of neurons). Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p < 0.05$. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **C**) Quantification of mean number of segmentations (sections) of neurites segmented per neuron. Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p < 0.05$. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **D**) Quantification of mean length of segmentations (sections) of neurites segmented per neuron. Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p <$ 0.05. Shared control estimation plot: mean difference for comparisons against the shared control,

SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **B** to **C** have a supplementary plot of all data grouped as PD-vulnerable and PD-resilient, where an independent 2-group Mann-Whitney U Test was performed, and an asterisk denotes $p<0.05$. Precise values can be found in the supplementary tables alongside unpaired Gardner-Altman two group estimation plots.

PD-vulnerable neurons have a higher proportion of varicosities that are positive for Syt1.

Neurotransmitter release sites are known to represent sites of high energy consumption in neurons (Pulido & Ryan, 2021). As such, we hypothesize that their density could represent a defining characteristic of vulnerable neurons in PD. We therefore estimated the density of potential neurotransmitter release sites (varicosities) along the axonal domains of these projecting neurons (**Figure 27A, 27B**). Following 10 DIV, we segmented probable varicosities, identified them based on morphology and dimensions, and evaluated the presence of synaptotagmin1 (Syt1), a calcium sensor of exocytosis that is critical for neurotransmitter release in DA neurons (Banerjee et al., 2020; Delignat-Lavaud et al., 2021; Mendez et al., 2011), as an index of release-competent terminals. Strikingly, we find that PD-vulnerable neurons have a significantly higher proportion of varicosities that are positive for Syt1 (**Figure 28A, 28B**, **Figure 7-1**), suggesting that these neurons have a higher proportion of active neurotransmitter release sites. Further examination of axonal varicosity density, calculated as inter-varicosity distance and density per unit length of axon, revealed that there were no major differences between PD-vulnerable and PD-resilient neurons, with an inter-varicose distance in the range ~2-4μm (**Figure 28C**). Raphe serotonin and hypoglossal cholinergic neurons nonetheless had slightly higher inter-varicose distances, while DMV and hypoglossal cholinergic neurons had a higher density of varicosities per unit length (**Figure 28C, 8D**). (Unpaired mean difference of DMV (n = 12) minus SNc (n = 12) 45.6 [95CI 34.2; 72.2]) and XII neurons (Unpaired mean difference of XII (n = 12) minus SNc (n = 12) 33.2 [95CI 24.8; 47.4]). Together, these results provide support for the hypothesis that a distinguishing feature of vulnerable neurons is having an axonal arbor endowed with a high proportion of active neurotransmitter release sites, presumably linked with higher bioenergetic requirements, placing a larger load on the neuron's mitochondrial network, that is equally dense across neuron types (**Figure 24D**).

Figure 7

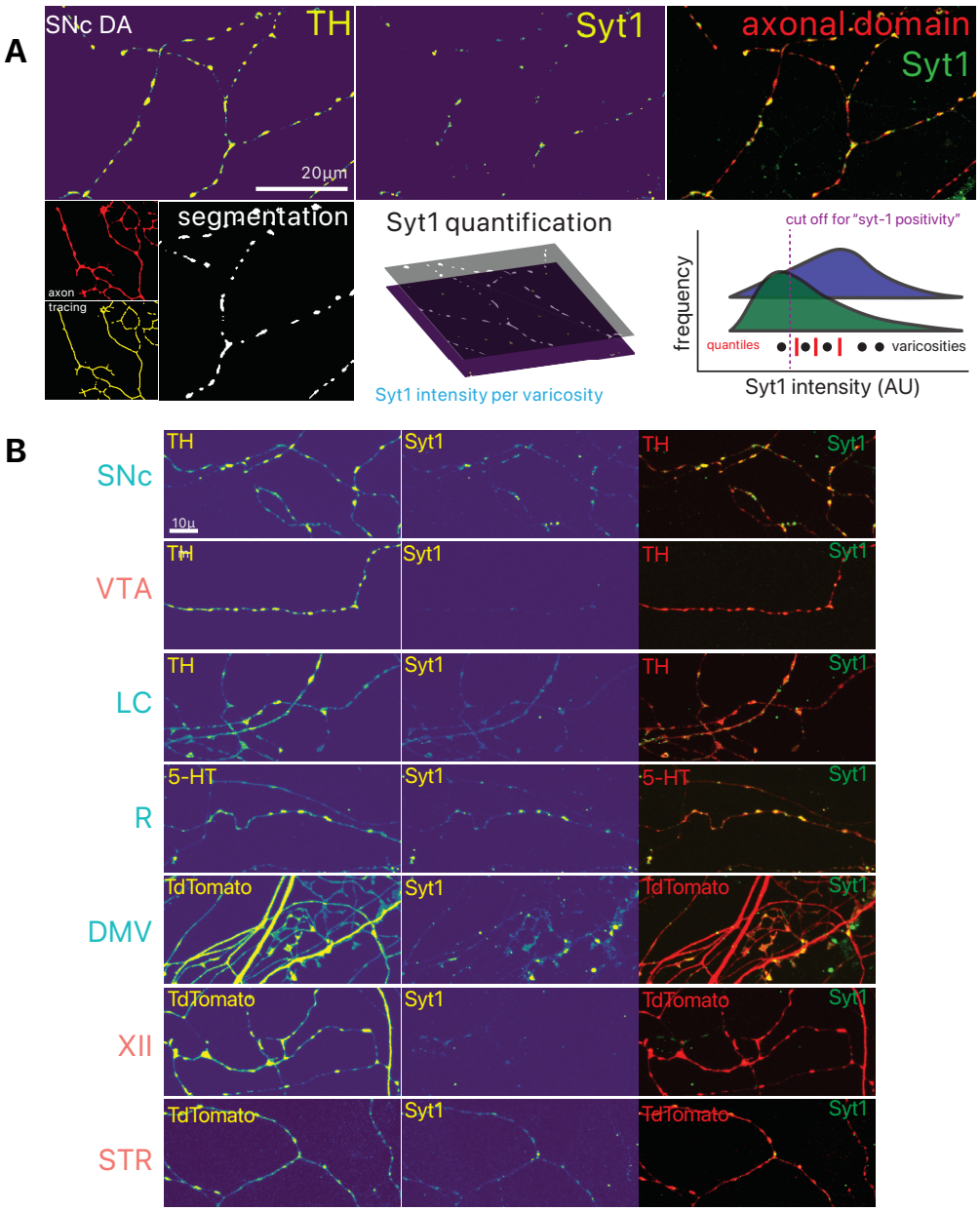


Figure 27 Revealing the proportion of varicosities that are positive for Syt1

A) Overview of image analysis for quantification of Syt1 positivity of varicosities, and distribution of Syt1 intensity within varicosities (bottom right). **B**) Photomicrographs of axonal fields of neurons, with their neurochemical marker and Syt1 immunocytochemistry.

Figure 8

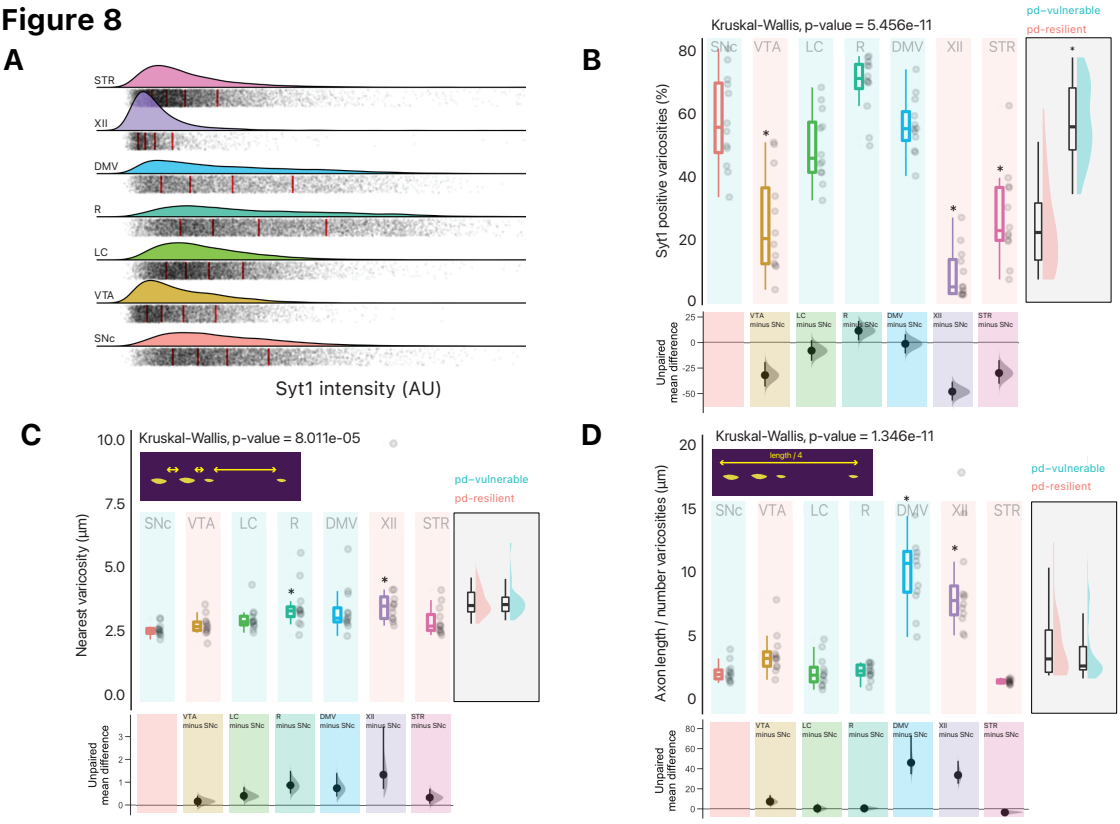


Figure 28 PD-vulnerable neurons have a higher proportion of varicosities that are positive for Syt1

A) Density plot of Syt1 intensity (arbitrary units (AU) - fluorescence) in all segmented varicosities included for analysis (red line indicate quintiles). **B**) Quantification of proportion of varicosities that are positive for Syt1. Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p < 0.05$. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **C**) Quantification of intervaricose distance (nearest neighbour analysis of segmentations). Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p < 0.05$. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **D**) Mean number of varicosities per unit length of axonal domain. Box and whiskers plots, in the style of Tukey, where we indicate the median value, and the lower and upper hinges correspond to the first and third quartiles, Kruskal-Wallis multiple comparison, p-values adjusted with the Bonferroni method, * = $p < 0.05$. Shared control estimation plot: mean difference for comparisons against the shared control, SNc, using Data Analysis with Bootstrap Estimation, with 5000 bootstrap resamples. All confidence intervals are bias-corrected and accelerated. **B** to **C** have a supplementary plot of all data grouped as PD-vulnerable and PD-resilient, where an independent 2-group Mann-Whitney U Test was performed, and an asterisk denotes $p<0.05$.

Precise values can be found in the supplementary tables alongside unpaired Gardner-Altman two group estimation plots.

13.06 Discussion

To our knowledge, this is the first study to compare the intrinsic vulnerability and morphological characteristics of several neuronal populations suspected to be vulnerable in PD, to others considered as more resilient in this disorder. Despite the obvious caveats associated with the use of *in vitro* models, the results of this study show that PD-vulnerable neurons, except for cholinergic DMV neurons, are more vulnerable to oxidative stress induced by hydrogen peroxide: a cellular stress model that is relevant in the context of the large body of work linking oxidative stress to cell loss in PD (El Kodsi et al., 2020; Jenner, 2003; Monzani et al., 2019). We also find that vulnerable neurons are endowed with a broad axonal arbor that bears a higher proportion of syt1-positive axonal varicosities. Broadly, our work supports a model proposing that a large and highly arborized axonal domain, coupled with a dense network of active neurotransmitter release sites render projection neuromodulatory neurons vulnerable due to such characteristics being linked to a high mitochondrial-dependent bioenergetic burden and elevated sensitivity to extra-homeostatic conditions.

PD-vulnerable neurons are more vulnerable to cell stress induced by hydrogen peroxide

As hypothesised, PD-vulnerable neurons were as a class, most vulnerable to the hydrogen peroxide cell stress assay. This finding and in particular the differential vulnerability of SNc and VTA DA neurons is in line with previous work (Mosharov et al., 2009; Pacelli et al., 2015) demonstrating increased vulnerability of SNc DA neurons to both hydrogen peroxide and MPP+. The finding that cholinergic DMV neurons were relatively resilient to our neurotoxicity assay is somewhat surprizing in light of previous data suggesting that these neurons are affected in PD. However, it is also possible that DMV neurons are vulnerable in PD via mechanisms that are not directly related to oxidative stress, such as through α-synuclein-dependent mechanisms (Chiu et al., 2021). However, given the very limited data derived from stereological counting methods validating whether DMV cholinergic neurons do, in fact, degenerate in PD (Giguère et al., 2018), it remains possible that our observation is explained by the fact that DMV cholinergic neurons are not particularly vulnerable in PD (Kalaitzakis et al., 2008). To strengthen these assertations, within the context of this experimental paradigm, it may be pertinent in future studies to use other cellular stress assays, such as α-synuclein overexpression or α-synuclein fibrils.

ROS production was not significantly different across neuron types

Using the mitochondrially-targeted ROS sensor mito-roGFP, we detected relatively similar levels of basal ROS production across all neuron types examined. We also found a very large spread of relative baseline oxidation status in the axonal and somatodendritic compartments of neurons (**Figure 4C**). The lack of significant difference between neuron types may be a bit surprizing considering that basal ROS production has been found to be significantly higher in SNc compared to VTA DA neurons both *in vitro* and *in vivo* (Guzman et al., 2010; Pacelli et al., 2015; Sanchez–Padilla et al., 2014) — as well as in LC (Sanchez–Padilla et al., 2014) and DMV neurons (Goldberg et al., 2012). One possibility is that *in vitro*, the lack of a sufficient level of synaptic inputs severely limits the firing frequency of these neurons and thus the level of metabolic activity and energy needs. Additional experiments driving neuronal firing pharmacologically or optogenetically would be required to further test this hypothesis. The use of a cell-wide ROS sensor such as DHE, as previously used (Pacelli et al., 2015), would also help to assess ROS production deriving from other sources in addition to mitochondrial activity. Strikingly, we also made the observation that, in our *in vitro* system, the distance between mitochondria along the axonal domain was consistent across neuron types (**Figure 24D**). This observation suggests that vulnerable neurons with highly complex axonal arbours may have a density of mitochondria along their axons that is comparable to that of simpler, more resilient neurons. And therefore, their higher level of vulnerability may not simply derive from a higher density of mitochondria. It remains that given the differential vulnerability to hydrogen peroxide detected in the present study, it can be assumed that cell-autonomous developmental differences between these neurons are present in the *in vitro* postnatal culture model used.

The complexity and activity of the axonal domain may be a key component of what renders a neuron vulnerable

A striking observation arising from the present study is the finding that globally, the neuronal populations that were most vulnerable to hydrogen peroxide neurotoxicity (SNc DA neurons, Raphe serotonin neurons and LC noradrenergic neurons) were also the ones with the largest axonal domain, and most robustly, with the largest proportion of syt1-positive axonal varicosities. These findings are in line with the hypothesis that at least in part, the elevated vulnerability of long-range projection neuromodulatory neurons is linked to the very large energetic requirements of their large numbers of neurotransmitter release sites found along their vast axonal domain. This conclusion is compatible with the hypothesis that processes occurring in axon terminals, and in particular vesicular neurotransmitter packaging, is particularly costly for neurons in terms of energy requirements (Pulido & Ryan, 2021).

One relative “anomaly” in the present data set is the surprising resilience of cholinergic DMV neurons, especially considering their large axonal domain and large proportion of syt1-positive release sites. Perhaps, ChAT+ DMV neurons are significantly less vulnerable to hydrogen peroxide as they develop less

complex – albeit very long – axonal arborizations. These arborization, in turn, contain far fewer varicosities per length of axon, despite having a similar proportion of varicosities that are active. Although we did not verify this in the present study, it can be reliably assumed that the presence of syt1 in varicosities is a reliable indicator of release site functionality (Brose et al., 1992; Ducrot et al., 2021). One possibility is that since DMV neurons project in large part to regions outside of the brain, the culture conditions used in the present study did not allow these neurons to get sufficient access to factors secreted by target cells in the peripheral nervous system and that are required to maintain these neurons' excitability and normal baseline activity. Previous work has provided support for the role of target cells in regulating the proportion of axonal varicosities formed by SNc and VTA DA neurons (Ducrot et al., 2021). Furthermore, there is a vast literature on the role of extracellular signals regulating axonal development (Bilimoria & Bonni, 2013). Complementary electrophysiological recordings coupled to the use of genetically encoded sensors of activity-dependent vesicular cycling would help to examine this possibility further. It remains important to note that in the *in vitro* context used in the present study, all of the examined long-range projection neurons nonetheless developed a very large and branched axonal domain, orders of magnitude larger than most other neuron types under similar conditions, supporting the hypothesis that such exuberant axonal growth is driven by an intrinsic developmental program.

13.07 Conclusion

Taken together, the results presented in the present study provide further support for the general relevance of the hypothesis that a key component of the selective vulnerability of neurons in PD (Wong et al., 2019) takes its origin in the large axonal domain of long-range projection neurons. We conclude that more efforts are now needed to better understand the bioenergetic challenges imposed by having a highly branched axonal domain endowed with a large number of active neurotransmitter release sites. In addition to the outright energetic demands imposed by such an axonal domain, it is intriguing to consider the possibility that such features may confer a massive demand on the endolysosomal system where, notably 12 out of 23 PARK genes (to denote their putative link to PD) are involved (Vidyadhara et al., 2019).

13.08 References

Alberico, S. L., Cassell, M. D., & Narayanan, N. S. (2015). The Vulnerable Ventral Tegmental Area in Parkinson's Disease. Basal Ganglia, 5(2–3), 51–55. https://doi.org/10.1016/j.baga.2015.06.001

Banerjee, A., Lee, J., Nemcova, P., Liu, C., & Kaeser, P. S. (2020). Synaptotagmin-1 is the Ca2+ sensor for fast striatal dopamine release. ELife, 9. https://doi.org/10.7554/eLife.58359

Bilimoria, P. M., & Bonni, A. (2013). Molecular Control of Axon Branching. The Neuroscientist, 19(1), 16–24. https://doi.org/10.1177/1073858411426201

Bolam, J. P., & Pissadaki, E. K. (2012). Living on the edge with too many mouths to feed: Why dopamine neurons die. Movement Disorders, 27(12), 1478–1483. https://doi.org/10.1002/mds.25135

Brose, N., Petrenko, A. G., Südhof, T. C., & Jahn, R. (1992). Synaptotagmin: A Calcium Sensor on the Synaptic Vesicle Surface. Science, 256(5059), 1021–1025.

Canadian Council on Animal Care. (1993). Guide to the care and use of experimental animals. Vol. 1. Vol. 1. Canadian Council on Animal Care.

Chiu, W.-H., Kovacheva, L., Musgrove, R. E., Arien-Zakay, H., Koprich, J. B., Brotchie, J. M., Yaka, R., Ben-Zvi, D., Hanani, M., Roeper, J., & Goldberg, J. A. (2021). α-Synuclein–induced Kv4 channelopathy in mouse vagal motoneurons drives nonmotor parkinsonian symptoms. Science Advances, 7(11), eabd3994. https://doi.org/10.1126/sciadv.abd3994

Connolly, N. M. C., Theurey, P., Adam-Vizi, V., Bazan, N. G., Bernardi, P., Bolaños, J. P., Culmsee, C., Dawson, V. L., Deshmukh, M., Duchen, M. R., Düssmann, H., Fiskum, G., Galindo, M. F., Hardingham, G. E., Hardwick, J. M., Jekabsons, M. B., Jonas, E. A., Jordán, J., Lipton, S. A., … Prehn, J. H. M. (2018). Guidelines on experimental methods to assess mitochondrial dysfunction in cellular models of neurodegenerative diseases. Cell Death & Differentiation, 25(3), 542–572. https://doi.org/10.1038/s41418-017-0020-4

Data Analysis using Bootstrap-Coupled Estimation [R package dabestr version 0.3.0]. (2020, July 13). https://CRAN.R-project.org/package=dabestr

Delignat-Lavaud, B., Kano, J., Ducrot, C., Massé, I., Mukherjee, S., Giguère, N., Moquin, L., Lévesque, C., Nanni, S. B., Bourque, M.-J., Rosa-Neto, P., Lévesque, D., Beaumont, L. D., & Trudeau, L.-É. (2021). The calcium sensor synaptotagmin-1 is critical for phasic axonal dopamine release in the striatum and mesencephalon, but is dispensable for basic motor behaviors in mice. BioRxiv, 2021.09.15.460511. https://doi.org/10.1101/2021.09.15.460511

Ducrot, C., Bourque, M.-J., Delmas, C. V., Racine, A.-S., Guadarrama Bello, D., Delignat-Lavaud, B., Domenic Lycas, M., Fallon, A., Michaud-Tardif, C., Burke Nanni, S., & others. (2021). Dopaminergic neurons establish a distinctive axonal arbor with a majority of non-synaptic terminals. The FASEB Journal, 35(8), e21791.

El Kodsi, D. N., Tokarew, J. M., Sengupta, R., Lengacher, N. A., Ng, A. C., Boston, H., Jiang, Q., Palmberg, C., Pileggi, C., & Shutinoski, B. (2020). Parkinson disease-linked parkin mediates redox reactions that lower oxidative stress in mammalian brain. BioRxiv. https://doi.org/10.1101/2020.04.26.062380

Espay, A. J., & Lang, A. E. (2018). Parkinson Diseases in the 2020s and Beyond: Replacing Clinico-Pathologic Convergence With Systems Biology Divergence. Journal of Parkinson's Disease, 8(s1), S59–S64. https://doi.org/10.3233/JPD-181465

Fasano, C., Thibault, D., & Trudeau, L.-É. (2008). Culture of Postnatal Mesencephalic Dopamine Neurons on an Astrocyte Monolayer. Current Protocols in Neuroscience, 44(1), 3.21.1-3.21.19. https://doi.org/10.1002/0471142301.ns0321s44

Giguère, N., Burke Nanni, S., & Trudeau, L.-E. (2018). On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease. Frontiers in Neurology, 9, 455. https://doi.org/10.3389/fneur.2018.00455

Giguère, N., Delignat-Lavaud, B., Herborg, F., Voisin, A., Li, Y., Jacquemet, V., Anand-Srivastava, M., Gether, U., Giros, B., & Trudeau, L.-É. (2019). Increased vulnerability of nigral dopamine neurons after expansion of their axonal arborization size through D2 dopamine receptor

conditional knockout. PLOS Genetics, 15(8), e1008352. https://doi.org/10.1371/journal.pgen.1008352

Goedert, M., Spillantini, M. G., Del Tredici, K., & Braak, H. (2013). 100 years of Lewy pathology. Nature Reviews Neurology, 9(1), 13–24. https://doi.org/10.1038/nrneurol.2012.242

Goldberg, J. A., Guzman, J. N., Estep, C. M., Ilijic, E., Kondapalli, J., Sanchez-Padilla, J., & Surmeier, D. J. (2012). Calcium entry induces mitochondrial oxidant stress in vagal neurons at risk in Parkinson's disease. Nature Neuroscience, 15(10), 1414–1421. https://doi.org/10.1038/nn.3209

Gong, S., Doughty, M., Harbaugh, C. R., Cummins, A., Hatten, M. E., Heintz, N., & Gerfen, C. R. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 27(37), 9817–9823. https://doi.org/10.1523/JNEUROSCI.2707-07.2007

Guzman, J. N., Sanchez-Padilla, J., Wokosin, D., Kondapalli, J., Ilijic, E., Schumacker, P. T., & Surmeier, D. J. (2010). Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature, 468(7324), 696–700. https://doi.org/10.1038/nature09536

Hirsch, E. C., Graybiel, A. M., Duyckaerts, C., & Javoy-Agid, F. (1987). Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. Proceedings of the National Academy of Sciences, 84(16), 5976–5980. https://doi.org/10.1073/pnas.84.16.5976

Ho, J., Tumkaya, T., Aryal, S., Choi, H., & Claridge-Chang, A. (2019). Moving beyond P values: Data analysis with estimation graphics. Nature Methods, 16(7), 565–566. https://doi.org/10.1038/s41592-019-0470-3

Huynh, B., Fu, Y., Kirik, D., Shine, J. M., & Halliday, G. M. (2021). Comparison of Locus Coeruleus Pathology with Nigral and Forebrain Pathology in Parkinson's Disease. Movement Disorders. https://doi.org/10.1002/mds.28615

ISH Data: Allen Brain Atlas: Developing Mouse Brain. (2008). https://developingmouse.brain-map.org/

Jenner, P. (2003). Oxidative stress in Parkinson's disease. Annals of Neurology, 53(S3), S26–S38. https://doi.org/10.1002/ana.10483

Kalaitzakis, M. E., Graeber, M. B., Gentleman, S. M., & Pearce, R. K. B. (2008). The dorsal motor nucleus of the vagus is not an obligatory trigger site of Parkinson's disease: A critical analysis of alpha-synuclein staging. Neuropathology and Applied Neurobiology, 34(3), 284–295. https://doi.org/10.1111/j.1365-2990.2007.00923.x

Lehtonen, Š., Sonninen, T.-M., Wojciechowski, S., Goldsteins, G., & Koistinaho, J. (2019). Dysfunction of Cellular Proteostasis in Parkinson's Disease. Frontiers in Neuroscience, 13. https://doi.org/10.3389/fnins.2019.00457

Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A. F., Boguski, M. S., Brockway, K. S., Byrnes, E. J., Chen, L., Chen, L., Chen, T.-M., Chi Chin, M., Chong, J., Crook, B. E., Czaplinska, A., Dang, C. N., Datta, S., … Jones, A. R. (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature, 445(7124), 168–176. https://doi.org/10.1038/nature05453

Lieberman, O. J., Choi, S. J., Kanter, E., Saverchenko, A., Frier, M. D., Fiore, G. M., Wu, M., Kondapalli, J., Zampese, E., Surmeier, D. J., Sulzer, D., & Mosharov, E. V. (2017). α-Synuclein-Dependent Calcium Entry Underlies Differential Sensitivity of Cultured SN and VTA Dopaminergic Neurons to a Parkinsonian Neurotoxin. ENeuro, 4(6), ENEURO.0167-17.2017. https://doi.org/10.1523/ENEURO.0167-17.2017

Lunt, W., Shah, M., Sang, J., Choi, J., Burke, S., Higgins, J., & Skene, N. (2021). Neurochemical identity and anatomy of cell loss in Parkinson's disease, and the clinical data available for correlation of progression: A systematic review and meta-analysis. https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021265515

Matsushita, N., Okada, H., Yasoshima, Y., Takahashi, K., Kiuchi, K., & Kobayashi, K. (2002). Dynamics of tyrosine hydroxylase promoter activity during midbrain dopaminergic neuron development. Journal of Neurochemistry, 82(2), 295–304. https://doi.org/10.1046/j.1471-4159.2002.00972.x

Mendez, J. A., Bourque, M.-J., Fasano, C., Kortleven, C., & Trudeau, L.-E. (2011). Somatodendritic Dopamine Release Requires Synaptotagmin 4 and 7 and the Participation of Voltage-gated Calcium Channels*. Journal of Biological Chemistry, 286(27), 23928–23937. https://doi.org/10.1074/jbc.M111.218032

Monzani, E., Nicolis, S., Dell'Acqua, S., Capucciati, A., Bacchella, C., Zucca, F. A., Mosharov, E. V., Sulzer, D., Zecca, L., & Casella, L. (2019). Dopamine, oxidative stress and protein–quinone modifications in Parkinson's and other neurodegenerative diseases. Angewandte Chemie International Edition, 58(20), 6512–6527. https://doi.org/10.1002/anie.201811122

Mosharov, E. V., Larsen, K. E., Kanter, E., Phillips, K. A., Wilson, K., Schmitz, Y., Krantz, D. E., Kobayashi, K., Edwards, R. H., & Sulzer, D. (2009). Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. Neuron, 62(2), 218–229. https://doi.org/10.1016/j.neuron.2009.01.033

Müller, K. (2020, December 13). A Simpler Way to Find Your Files [R package here version 1.0.1]. https://CRAN.R-project.org/package=here

Nicholas Sofroniew, Talley Lambert, Kira Evans, Juan Nunez-Iglesias, Philip Winston, Grzegorz Bokota, Kevin Yamauchi, Ahmet Can Solak, ziyangczi, Gonzalo Peña-Castellanos, Matthias Bussonnier, Genevieve Buckley, Draga Doncila Pop, Pam, alisterburt, Volker Hilsenstein, Tony Tung, Hector, Jeremy Freeman, … Abigail McGovern. (2021). napari/napari: 0.4.8rc2. Zenodo. https://doi.org/10.5281/zenodo.4747712

Pacelli, C., Giguère, N., Bourque, M.-J., Lévesque, M., Slack, R. S., & Trudeau, L.-É. (2015). Elevated Mitochondrial Bioenergetics and Axonal Arborization Size Are Key Contributors to the Vulnerability of Dopamine Neurons. Current Biology, 25(18), 2349–2360. https://doi.org/10.1016/j.cub.2015.07.050

Parent, M., & Parent, A. (2006). Single-axon tracing study of corticostriatal projections arising from primary motor cortex in primates. Journal of Comparative Neurology, 496(2), 202–213. https://doi.org/10.1002/cne.20925

Pedersen, T. L. (2021, March 5). Accelerating "ggplot2" [R package ggforce version 0.3.3]. https://CRAN.R-project.org/package=ggforce

Pissadaki, E. K., & Bolam, J. P. (2013). The energy cost of action potential propagation in dopamine neurons: Clues to susceptibility in Parkinson's disease. Frontiers in Computational Neuroscience, 7. https://doi.org/10.3389/fncom.2013.00013

R: The r project for statistical computing. (n.d.). Retrieved June 3, 2021, from https://www.r-project.org/

Rossi, J., Balthasar, N., Olson, D., Scott, M., Berglund, E., Lee, C. E., Choi, M. J., Lauzon, D., Lowell, B. B., & Elmquist, J. K. (2011). Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metabolism, 13(2), 195–204. https://doi.org/10.1016/j.cmet.2011.01.010

RStudio | Open source & professional software for data science teams. (n.d.). Retrieved June 3, 2021, from https://rstudio.com/

Sanchez–Padilla, J., Guzman, J. N., Ilijic, E., Kondapalli, J., Galtieri, D. J., Yang, B., Schieber, S., Oertel, W., Wokosin, D., Schumacker, P. T., & Surmeier, D. J. (2014). Mitochondrial oxidant stress in locus coeruleus is regulated by activity and nitric oxide synthase. Nature Neuroscience, 17(6), 832–840. https://doi.org/10.1038/nn.3717

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089

Simple fisheries stock assessment methods [r package fsa version 0. 8. 32]. (2021, January 15). https://CRAN.R-project.org/package=FSA

Slowikowski, K. (2021, January 15). Automatically Position Non-Overlapping Text Labels with "ggplot2" [R package ggrepel version 0.9.1]. https://CRAN.R-project.org/package=ggrepel

Surmeier, D. J., Obeso, J. A., & Halliday, G. M. (2017a). Selective neuronal vulnerability in Parkinson disease. Nature Reviews Neuroscience, 18(2), 101–113. https://doi.org/10.1038/nrn.2016.178

Surmeier, D. J., Obeso, J. A., & Halliday, G. M. (2017b). Parkinson's Disease Is Not Simply a Prion Disorder. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 37(41), 9799–9807. https://doi.org/10.1523/JNEUROSCI.1787-16.2017

Thévenaz, P., Ruttimann, U. E., & Unser, M. (1998). A pyramid approach to subpixel registration based on intensity. IEEE Transactions on Image Processing: A Publication of the IEEE Signal Processing Society, 7(1), 27–41. https://doi.org/10.1109/83.650848

Tubert, C., Galtieri, D., & Surmeier, D. J. (2019). The pedunclopontine nucleus and Parkinson's disease. Neurobiology of Disease, 128, 3–8. https://doi.org/10/gmvwvd

Vidyadhara, D. J., Lee, J. E., & Chandra, S. S. (2019). Role of the endolysosomal system in Parkinson's disease. Journal of Neurochemistry, 150(5), 487–506. https://doi.org/10.1111/jnc.14820

Wickham, H. (2021, March 3). Tidy Messy Data [R package tidyr version 1.1.3]. https://CRAN.R-project.org/package=tidyr

Wilke, C. O. (2021, January 8). Ridgeline Plots in "ggplot2" [R package ggridges version 0.5.3]. https://CRAN.R-project.org/package=ggridges

Wong, Y. C., Luk, K., Purtell, K., Burke Nanni, S., Stoessl, A. J., Trudeau, L., Yue, Z., Krainc, D., Oertel, W., Obeso, J. A., & Volpicelli-Daley, L. A. (2019). Neuronal vulnerability in Parkinson disease: Should the focus be on axons and synaptic terminals? Movement Disorders, 34(10), 1406–1422. https://doi.org/10.1002/mds.27823

14 Chapter 5 — Simple and reproducible methods for counting primary projecting neurons *in vitro*

Overview

The quantification of cell numbers in *in vitro* experiments can be automated using available, and relatively simple, existing tools. However, for projecting neuromodulatory neurons, such as Substantia nigra pars compacta dopamine neurons, these methods are not able to accurately perform this task. In this manuscript we describe a simple, yet robust, imaging analysis pipeline that achieves ~90% accuracy, when compared to manual counting methods, in a cell stress assay. This image analysis pipeline is written in python and is available as an iPython Jupyter Notebook, enabling simple to deploy, and reproducible, quantifications of projecting neurons in *in vitro* assays.

Contributions

Samuel Burke: conception, data collection, analysis, writing of manuscript.

Alex Tchung: conception, data collection, analysis.

Louis-Eric Trudeau: providing resources, supervision, writing of manuscript.

Available: Will be posted as a pre-print, with the notebooks hosted on GitHub for access — and in preparation for publication.

Simple and reproducible methods for counting primary projecting neurons *in vitro*

Samuel Burke*, Alex Tchung*, and Louis-Eric Trudeau

* Contributed equally

14.01 Abstract

The quantification of cell numbers in *in vitro* experiments can be automated using available, and relatively simple, existing tools. However, for projecting neuromodulatory neurons, such as Substantia nigra pars compacta dopamine neurons, these methods are not able to accurately perform the same task. In these brief methods we describe a simple, yet robust, imaging analysis pipeline that achieves ~90% accuracy, when compared to manual counting methods, in a cell stress assay. This image analysis pipeline is written in python and is available as an iPython Jupyter Notebook, enabling simple to deploy, and reproducible, quantifications of projecting neurons in *in vitro* assays.

14.02 Introduction

The quantification of cell numbers in *in vitro* and *in vivo* experiments is a foundational variable in experimental biology. However, manually counting cells is a laborious task. Classically, the task will be achieved by either sitting at the microscope, or computer — with a counting-clicker (or equivalent) — for extended periods of time. With the advent of user-friendly automated imaging systems, relatively high throughput experiments are increasingly imaged for cell number quantification by scientists. With relatively little computational ability, most biologists can use software such as ImageJ (Schneider et al., 2012), CellProfiler (McQuin et al., 2018) or other similar products to achieve extremely accurate quantifications of cells that have simple morphological features such as stained nuclei. However, for neurons, and especially projecting neurons, this becomes a significant challenge. These types of cells have more complex morphologies, often with significant numbers of overlapping neurites, increasing the risk of errors in identifying and counting individual cells. In addition, such cell types have received less attention from software developers.

The current literature and documentation for methods for quantifying cell numbers are either limited to imaging segmentation tasks that are too simple or describing methods that require extensive knowledge in mathematics or computer programming. In the context of *in vitro* experiments, the quantification of number of nuclei can be achieved with very simple segmentation techniques. However, when working with tissue samples, the task becomes significantly more complex. Open competitions have allowed the open sourcing of machine learning and data science practitioners to apply their methods to these tasks and have

achieved very high accuracy in nuclei segmentation challenges (Caicedo et al., 2019). However, experimental biologist are faced with major challenges in the use of these methods.

For the more difficult task of automating the counting of neurons, multiple authors have described the application of state-of-the-art methods to achieve accurate quantifications (Iqbal et al., 2019; Tyson et al., 2020). More recently authors have also attempted to create deep learning solutions to the problem of counting neurons, that can be used as a plug-in in ImageJ. Albeit a valiant effort to "... present an ImageJ plugin that enables non-machine-learning experts to analyze their data with U-Net on either a local computer or a remote server/cloud service" (Falk et al., 2019), the implementation of such methods is still challenging for many biologists, and for *in vitro* contexts, perhaps unnecessarily complex.

Finally, a grossly underserved aspect of many of these more advanced methods is the pedagogical component and user-friendliness. To implement many of the existing methods, specific domain expertise — even the most basic — is required in the computational realm. One solution is for these methods and protocols to be contained within notebook interfaces such as Jupyter Notebooks (*Project Jupyter*, n.d.). In addition, using these formats for the sharing of methods may help instil a culture of open documentation and analysis.

In this work we describe simple to use and apply methods that allow biologist/neuroscientists to quantify the number of projecting neurons cultured in multi-well plates, as well as becoming familiar with the Jupyter notebook format. In this manuscript, we show that simple image processing can achieve ~90% accuracy in quantifying neuronal numbers *in vitro.*

14.03 Methods

Procedures with animals and their care were conducted in accordance with the Guide to care and use of Experimental Animals of the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). Experimental protocols were approved by the animal ethics committees of the Université de Montréal. Housing was at a constant temperature of 21°C and humidity of 60%, under a fixed 12-hour light / dark cycle, with food and water available ad libitum. Primary neuron cultures were prepared from dissections of P0 mice as described previously with slight modification (Fasano et al., 2008), and seeded into 96 well plates (µ-Plate 96 Well Black, ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized; Cat.No. 89626). Primary neuron cultures were from the study (Chapter 4, Burke et al., 2021) and random images were included in our training set from cultures prepared from DA neurons of the SN, noradrenergic neurons of the locus coeruleus (LC), serotonin neurons of the raphe nuclei (R), cholinergic neurons of the dorsal motor nucleus of the vagus (DMV), DA neurons of the VTA, cholinergic neurons of the hypoglossal nucleus (XII), and cholinergic interneurons of the dorsal striatum (STR). Neurons were

cultured and then treated with hydrogen peroxide at 0, 100, 150, and 200µM, at 10 days in vitro (DIV), and cells were fixed at 11 DIV. Immunocytochemistry was performed to reveal the neurochemical identity of interest (as described in our previous study Burke et al., 2021 and imaging were acquired on a Nikon Eclipse Ti2-E inverted microscope using a CFI Plan Apo Lambda 20X objective. Images were acquired and stored as single channel 16-bit TIFF images.

14.04 Image processing and neuron counting

To achieve simple segmentations of cell bodies to allow quantification of neurons numbers, raw images are processed using simple image processing algorithms available across a variety of image processing libraries. For these methods we use, Python (Van Rossum & Drake, 2009), iPython Jupyter Notebooks (Kluyver et al., 2016), NumPy (Harris et al., 2020), and the scikit-image library (Walt et al., 2014).

Image visualization

As a first step, images are opened and visualised with exposure being adjusted using two methods of automated exposure modification, to help visualisation and evaluation of subsequent image processing (Figure 29).

```
#open images using scikit-image and matplotlib
from skimage.io import imread

image = imread(address_to_image')
from skimage import exposure
equalized_data = exposure.equalize_hist(image)
gamma_modified= exposure.adjust_gamma(image, gamma=0.1)

#plot imported image
fig, ax = plt.subplots(1, 3, figsize=(30, 15))
ax[0].imshow(image, cmap='viridis')
ax[0].set_title('Original')
ax[1].imshow(equalized_data, cmap='viridis')
ax[1].set_title('Equalized')
ax[2].imshow(gamma_modified, cmap='viridis')
ax[2].set_title('Gamma Modified')
plt.show()
```

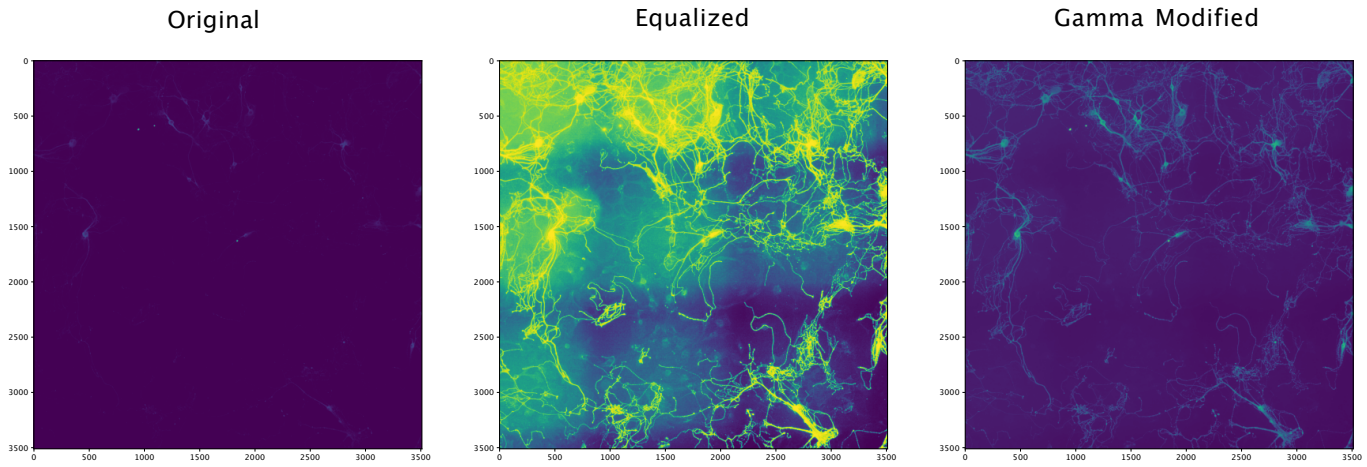


Figure 29 Image visualization. Modification of brightness and contrast using two exposure adjustment methods.

Image processing

To be able to count the number of neurons present in an image, we implemented a multi-step image processing pipeline that cleans and normalizes images, independent of sample preparation and image acquisition parameters (**Figure 30**). This is then followed by image segmentation to quantify the number of neurons present within each image.

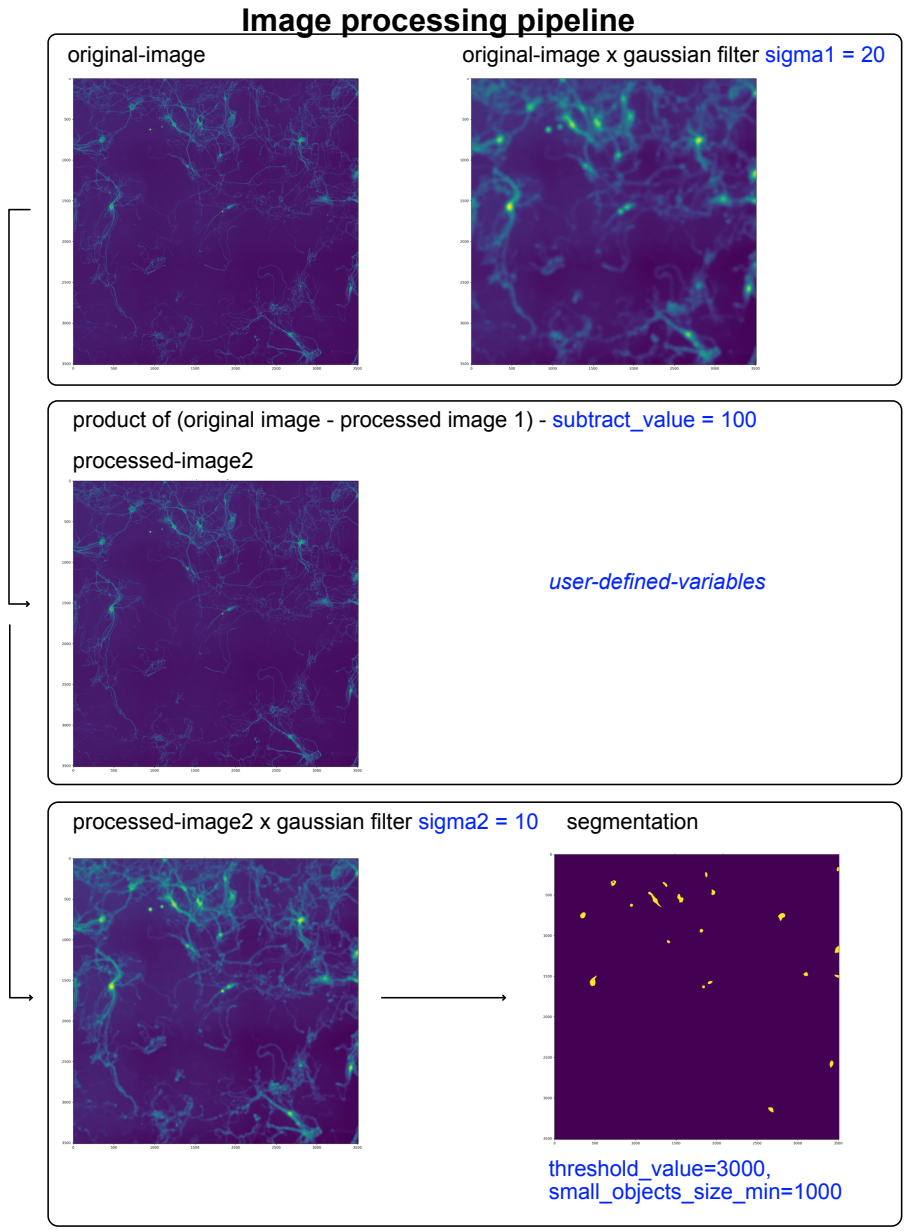


Figure 30 Image processing pipeline

A gaussian filter is applied to the raw image, with a user defined sigma. The product of a subtraction of this processed image is then subtracted (with an additional value added) from the raw image. Subsequently a second gaussian filter is applied, with a user defined sigma, and binarization of the image is achieved by manual thresholding with a minimum object size. The resulting image can then be used to quantify the number of neurons present in the image.

Five variables (which are pre-set) can be modified by the user for their use-case if segmentation requires optimization. These values within the notebook are:

```
# first sigma for gaussian filter
sigma1=20
# value to subtract from image
subtract_value=100
# sigma for second gaussian filter
sigma2=10
# you can get an idea for where this should be calling mean_threshold or
median_threshold
threshold_value=3000
# minimum size of objects to remove – this can help for small objects such
as dirt or noise
small_objects_size_min=1000
```

Within the notebook, the user can iterate across both sample images and modify the cell counting algorithm until accurate neuron segmentation is achieved. This is visualised by showing the original image, the segmentation product (**Figure 31**), and a composite image of segmentation and original image (**Figure 32**).

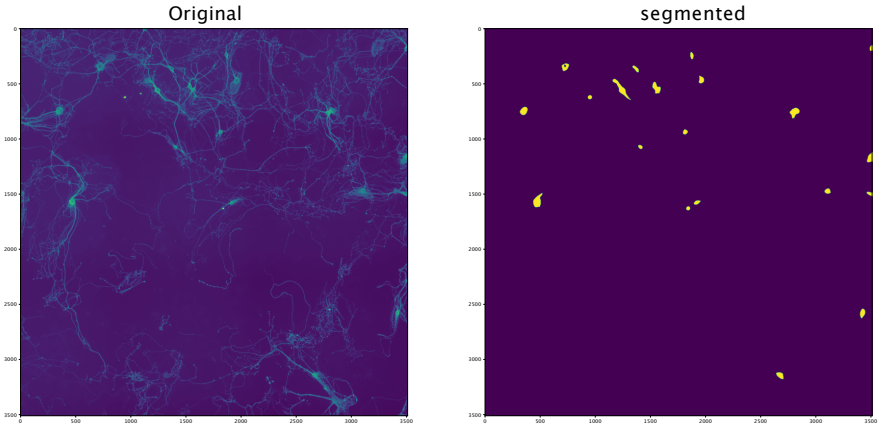


Figure 31 Result of segmentation

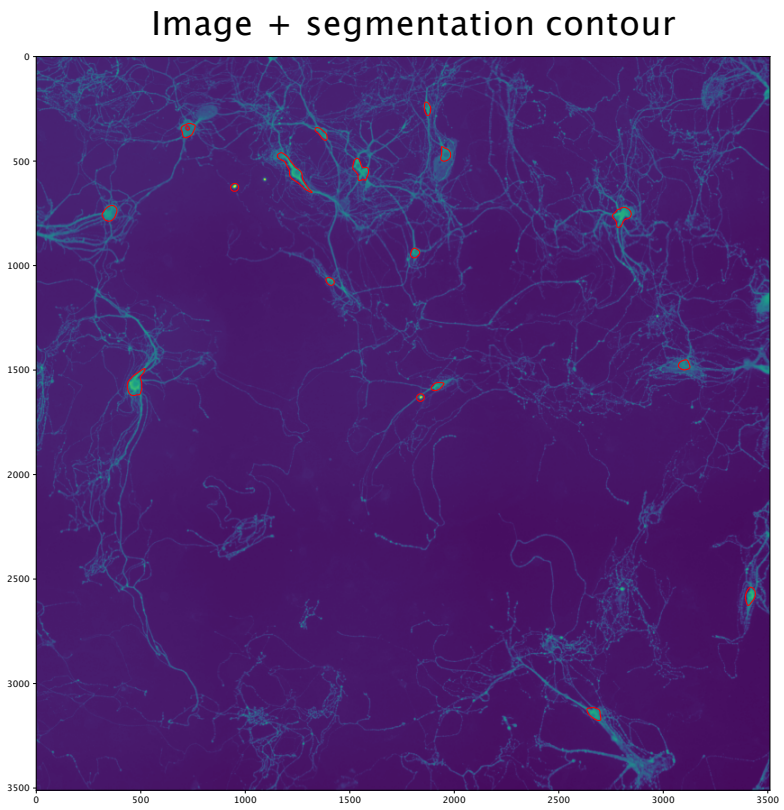


Figure 32 Segmentation of neurons for neuron counting

The results of image binarization, following the image analysis pipeline, can be visualized with the contours of segmented objects shown *in situ*, on the raw images. Note that objects on the of the image are not shown in this plot but are counted.

Once suitable parameters have been set, the user may execute the notebook to quantify neuron numbers found within images contained within a declared parent directory (**Figure 33**). To implement, the user needs only to point the notebook to the folder containing imaging data, by modifying the directory address:

```
folder = 'parent_directory_of_images'
```

Results of the count are saved as a .csv file within the notebook directory, ‘data_out’.

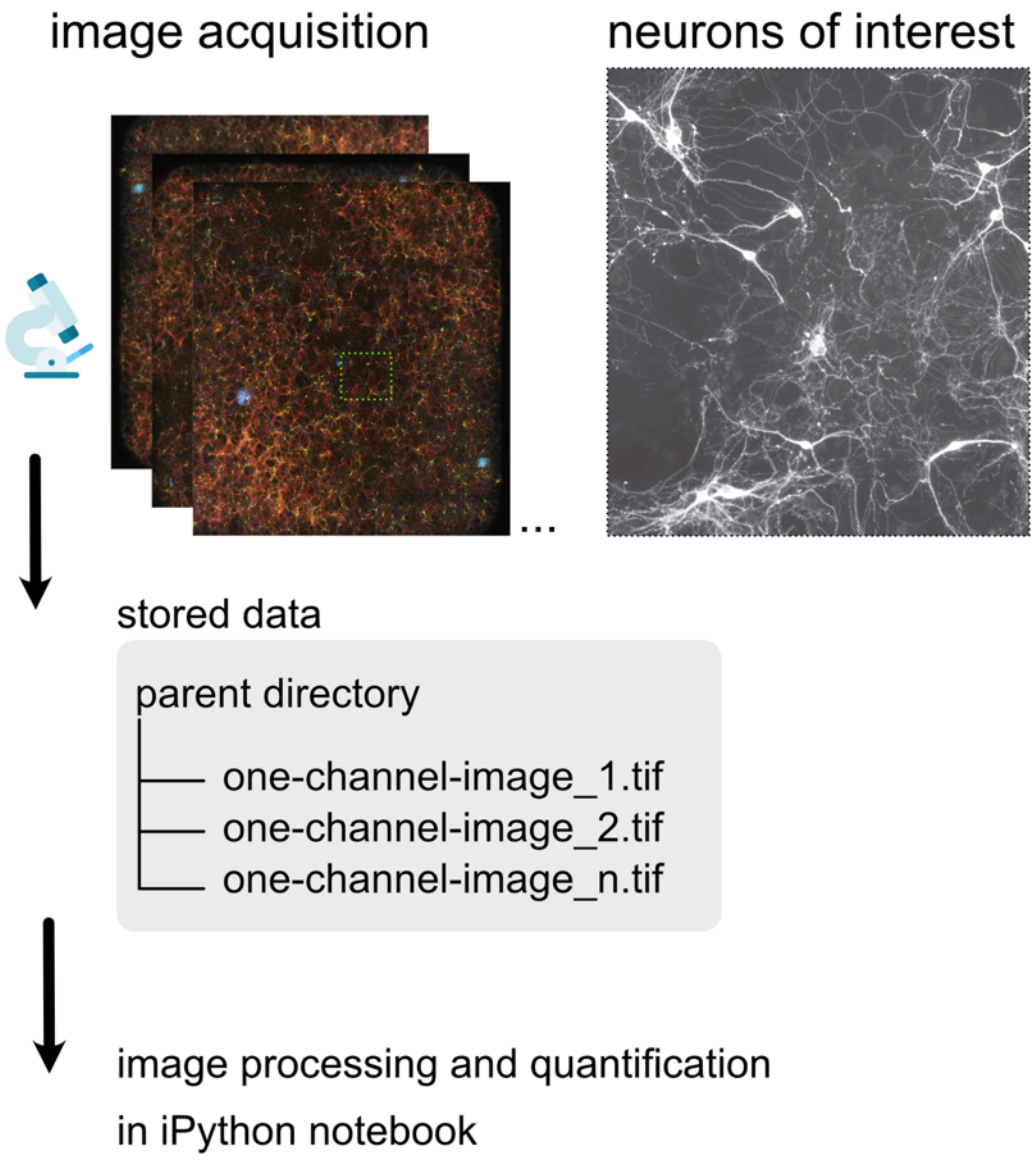


Figure 33 Overview of image processing for quantification of neuron numbers

14.05 Code required to execute count of neurons

The following code is the minimum amount of code required to execute a count of neurons, on a directory of images, where the resultant quantification is saved as a comma separated values (CSV) file. The code can be executed either as python file, or iPython Notebook. The pre-set values ("sigma1=30", "subtract_value=100", "sigma2=15", "threshold_value=3000", "small_objects_size_min=1000") work on the test data set, but may be modified for user-specific data sets.

```
import skimage.io as io
from skimage.measure import label
import os
from skimage import filters
from skimage.morphology import remove_small_objects
folder = 'parent_directory_of_images'
## ————————
## modify this section
sigma1=30
subtract_value=100
sigma2=15
threshold_value=3000 #which you can base around mean_threshold
small_objects_size_min=1000
## ————————
file_list = [f for f in os.listdir(folder) if f.endswith('.tif')]
#close number_of_neurons_list and file_name_list if open
if 'number_of_neurons_list' in locals():
    del number_of_neurons_list
if 'file_name_list' in locals():
    del file_name_list
#for loop to open each file in folder and run the function to count the
number of neurons
for file in file_list:
    image=io.imread(folder + file)
#mutlti step image processing with variables from above. Rinse and repieat
until satistified
    image = image - filters.gaussian(image, sigma1)
    image = (image - subtract_value).clip(min=0)
    image = filters.gaussian(image, sigma2)
    image = image > threshold_value
    image = remove_small_objects(image, min_size=small_objects_size_min)
#remove small objects - this minima is set to 1000, can be modified above
    #count number of objects segmented
    label_image = label(image)
    number_of_neurons = label_image.max()
    print(file)
```

```
print(' contains roughly ' + str(number_of_neurons) + ' neurons')
    #create list of number of neurons in each image
    #create list if doesnt exist
    if 'number_of_neurons_list' not in locals():
        number_of_neurons_list = []
    number_of_neurons_list.append(number_of_neurons)
    #create list of file names
    if 'file_name_list' not in locals():
        file_name_list = []
    file_name_list.append(file)
#create data frame with number of neurons and file name
import pandas as pd
#create data frame
df = pd.DataFrame({'file_name':file_name_list,
'number_of_neurons':number_of_neurons_list})
#sort df by file name alphabetically
df = df.sort_values(by='file_name')
#print data frame
print(df)
#save data frame as csv in current directory
df.to_csv('data_out/number_of_neurons.csv')
```

14.06 Performance on known data set

To evaluate the performance of the methods described here to quantify neuron numbers, random images were sampled from a dataset previously collected where projecting neurons were cultured and treated with the cell stressor hydrogen peroxide. These images were manually counted for ground truth neuron numbers and subsequently counted using our simple automated methods. We found that our automated methods achieved reliable estimates of neuron numbers (Pearson's correlation comparing automatic count vs manual count $R^2 = 0.901$, $p < 0.001$, slope = 1.1**, Figure 34**). Furthermore, we validated that there were no statistically significant differences between manual and automated counts for the mean values across the whole dataset (**Figure 35**, paired t-test assuming unequal variances, t stat: -0.95 p value: 0.34), as well as for individual concentrations of hydrogen peroxide (**Figure 36**, paired t-test assuming unequal variances; hydrogen peroxide = 0, t stat = 0.0, p = 1.1; hydrogen peroxide = 100, t stat = 0.97, p =

0.35; hydrogen peroxide = 150, t stat = 1.35, p = 0.199; hydrogen peroxide = 200, t stat = -1.59, p = 0.14) suggesting that these simple methods can be used to accurately quantify neuron numbers in assays designed to modify cell survival.

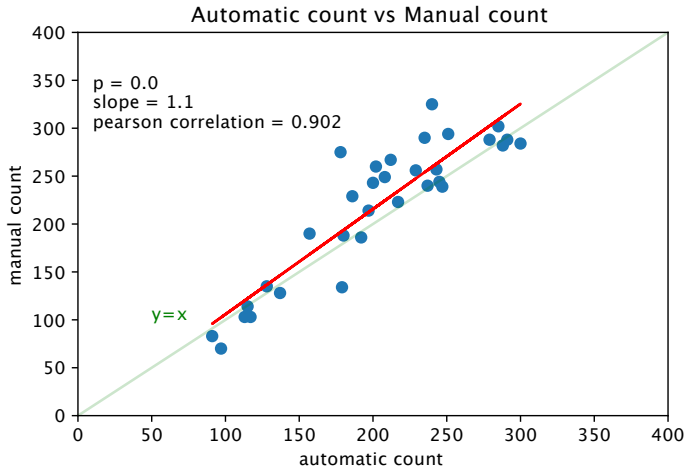


Figure 34 Automated vs manual counts

Pearson's $R^2 = 0.901$, $p < 0.001$, and slope = 1.1 with line of y = x plotted for refence. Red line is best fit.

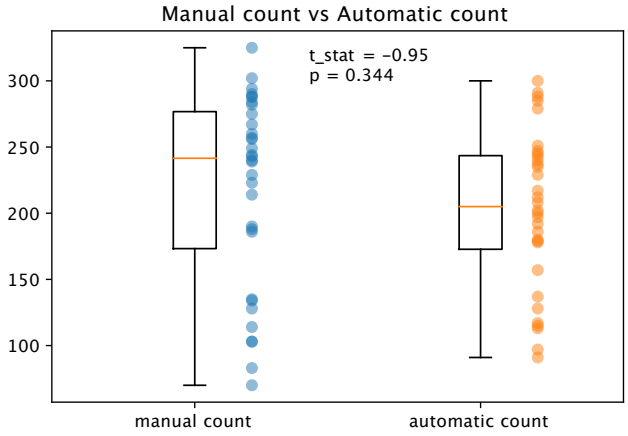


Figure 35 Comparing mean difference between manual and automated counts

Paired t-test assuming unequal variances, t stat: -0.954 p value: 0.344. Box and whisker plot: the box extends from the lower to upper quartile values of the data, with a line at the median. The whiskers extend from the box to show the range of the data. Flier points are those past the end of the whiskers.

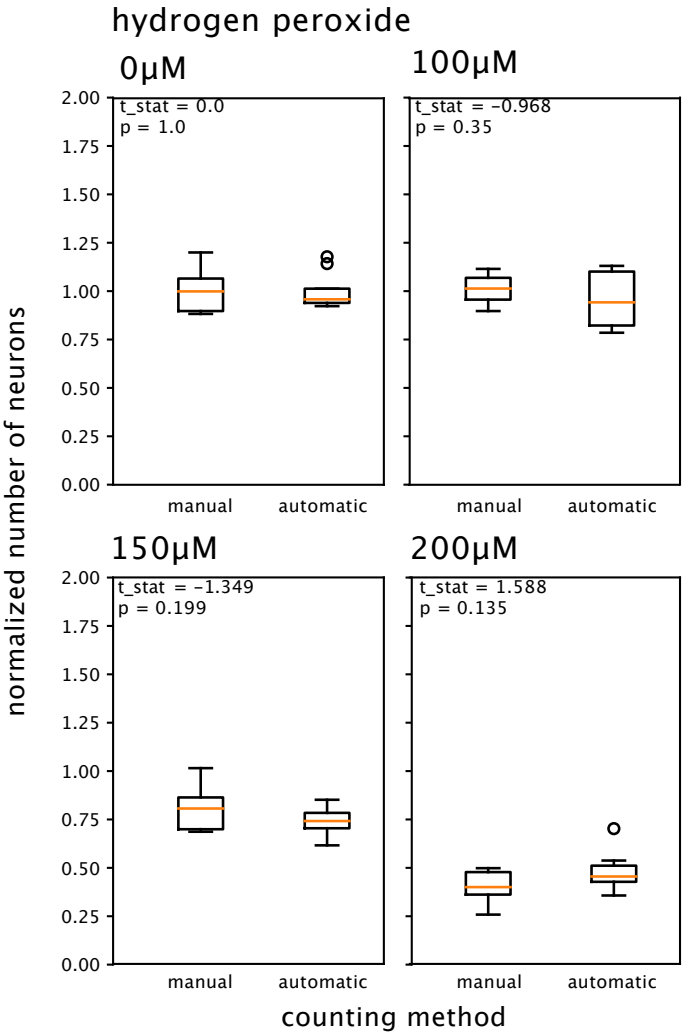


Figure 36 Comparing mean quantifications of counts for each concentration of hydrogen peroxide treatment

Paired t-test assuming unequal variances (results on plot). Box and whisker plot: the box extends from the lower to upper quartile values of the data, with a line at the median. The whiskers extend from the box to show the range of the data. Flier points are those past the end of the whiskers.

14.07 Conclusion

Though simple, the method described here allow for robust quantification of neuron numbers in primary cultures of projecting neurons cultured in multi-well plates. We show that the approach is reliable to quantify neuron numbers across a range of ~100-300 projecting neurons per well imaged, as well as for cells that have been treated with hydrogen peroxide. We find that there is a linear relationship between the manual and automatic counts, validating that the approach is robust enough to allow useful quantification

in *in vitro* assays. Using this method, biologists/neuroscientists will be able to produce reliable quantifications of neuron cell numbers in primary cultures that allow for robust sample sizes to be collected: previously a highly time-consuming task. Additionally, this method can be hosted and executed on Google Colab, allowing zero-set-up on a user's own machine (often a challenge for non-computational scientists): similarly, to other similar work (von Chamier et al., 2021). In addition, our performance, though not as high as other methods, reaches similar levels as deep learning approaches optimized for three-dimensional samples (Falk et al., 2019; Tyson et al., 2020). Finally, the notebook nature of this method allows for data analysis parameters to be saved for future reference, and to allow reproducibility of findings.

14.08 Bibliography

Caicedo, J. C., Goodman, A., Karhohs, K. W., Cimini, B. A., Ackerman, J., Haghighi, M., Heng, C., Becker, T., Doan, M., McQuin, C., Rohban, M., Singh, S., & Carpenter, A. E. (2019). Nucleus segmentation across imaging experiments: The 2018 Data Science Bowl. Nature Methods, 16(12), 1247–1253. https://doi.org/10.1038/s41592-019-0612-7

Falk, T., Mai, D., Bensch, R., Çiçek, Ö., Abdulkadir, A., Marrakchi, Y., Böhm, A., Deubner, J., Jäckel, Z., Seiwald, K., Dovzhenko, A., Tietz, O., Dal Bosco, C., Walsh, S., Saltukoglu, D., Tay, T. L., Prinz, M., Palme, K., Simons, M., … Ronneberger, O. (2019). U-Net: Deep learning for cell counting, detection, and morphometry. Nature Methods, 16(1), 67–70. https://doi.org/10.1038/s41592-018-0261-2

Fasano, C., Thibault, D., & Trudeau, L.-É. (2008). Culture of Postnatal Mesencephalic Dopamine Neurons on an Astrocyte Monolayer. Current Protocols in Neuroscience, 44(1), 3.21.1-3.21.19. https://doi.org/10.1002/0471142301.ns0321s44

Harris, C. R., Millman, K. J., Walt, S. J. van der, Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N. J., Kern, R., Picus, M., Hoyer, S., Kerkwijk, M. H. van, Brett, M., Haldane, A., Río, J. F. del, Wiebe, M., Peterson, P., … Oliphant, T. E. (2020). Array programming with NumPy. Nature, 585(7825), 357–362. https://doi.org/10.1038/s41586-020-2649-2

Iqbal, A., Sheikh, A., & Karayannis, T. (2019). DeNeRD: High-throughput detection of neurons for brain-wide analysis with deep learning. Scientific Reports, 9(1), 13828. https://doi.org/10.1038/s41598-019-50137-9

Iterator. (2021). In Wikipedia. https://en.wikipedia.org/w/index.php?title=Iterator&oldid=1022023119

Kluyver, T., Ragan-Kelley, B., Pérez, F., Granger, B., Bussonnier, M., Frederic, J., Kelley, K., Hamrick, J., Grout, J., Corlay, S., Ivanov, P., Avila, D., Abdalla, S., & Willing, C. (2016). Jupyter Notebooks – a publishing format for reproducible computational workflows. In F. Loizides & B. Schmidt (Eds.), Positioning and Power in Academic Publishing: Players, Agents and Agendas (pp. 87–90). IOS Press.

McQuin, C., Goodman, A., Chernyshev, V., Kamentsky, L., Cimini, B. A., Karhohs, K. W., Doan, M., Ding, L., Rafelski, S. M., Thirstrup, D., Wiegraebe, W., Singh, S., Becker, T., Caicedo, J. C., & Carpenter, A. E. (2018). CellProfiler 3.0: Next-generation image processing for biology. PLOS Biology, 16(7), e2005970. https://doi.org/10.1371/journal.pbio.2005970

Project Jupyter. (n.d.). Retrieved May 10, 2021, from https://www.jupyter.org

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089

Tyson, A. L., Rousseau, C. V., Niedworok, C. J., Keshavarzi, S., Tsitoura, C., Cossell, L., Strom, M., & Margrie, T. W. (2020). A deep learning algorithm for 3D cell detection in whole mouse brain image datasets [Preprint]. Neuroscience. https://doi.org/10.1101/2020.10.21.348771

Van Rossum, G., & Drake, F. L. (2009). Python 3 Reference Manual. CreateSpace.

Walt, S. van der, Schönberger, J. L., Nunez-Iglesias, J., Boulogne, F., Warner, J. D., Yager, N., Gouillart, E., & Yu, T. (2014). scikit-image: Image processing in Python. PeerJ, 2, e453. https://doi.org/10.7717/peerj.453

15 Chapter 6 — Discussion

15.01 General considerations

In Chapter 3, we end with the quote, "What if our understanding of Parkinson's disease is also impeding our ability to find cures? Could it be that generating hypotheses based on what we think we know, along with our rigid funding models, is making it nearly impossible to find what we really need to know?" (Espay & Stecher, 2020). Without the necessary depth for such important and large questions, I believe that this thesis begins to address some fundamental questions and assumptions generate the simple question: why are certain neurons affected in PD, and others are not?

Many elements of disease-centric research preclude this question. Basic, fundamental, science finds itself at the interface of biology and disease. Much of our funding is disease-centric, yet the knowledge required for answering questions about pathophysiology requires a solid understanding of physiology — a reference to compare to; where even here, the dichotomy is likely naïve. In the context of PD and the other neurodegenerative diseases, we aim to prevent and treat. Yet, we concurrently aim to understand why PD is happening, to understand which biological systems are in dysfunction, how these systems function normally, and how to intervene, or 'rescue', these yet-to-be-fully understood dysfunctional systems. I would argue that there is little acknowledgement of the overt challenge such an approach entails; how it may be "making it nearly impossible to find what we really need to know". Of course, I do believe that funding bodies are responsible for many of these issues, in part: they dangle the proverbial carrot, after all. I recognise that criticizing "translational impact" is relatively easy, and has been articulated for some time (Fang & Casadevall, 2010; Microbe, 2021; Seyhan, 2019; Szaszi, 2015).

I would argue that as scientists, however, how we describe and discuss our day-to-day observations is equally important. Not only must we verbalise and publish our valuing of basic science for the sake of basic science (*Curiosity Creates Cures: The Value and Impact of Basic Research*, n.d.), we should also believe it in such a manner that it guides our day-to-day. We may find ourselves exploring, for example, a specific aspect of DA neuron biology in the context of a mouses behaviour and find ourselves trying to *understand* what this means for PD or addiction/depression/schizophrenia in humans. Of course, there is a link. Of course, there are examples where rodent models have — serendipitously, I would argue — managed to capture key elements of biology and be transformative. However, I would suggest that these are exceptions that prove the rule. When we have a body of data from model systems, from a multitude of groups, that purport to explain a natural phenomenon causally, we can extrapolate and form hypotheses of

how these models translate into human context or apply more broadly to biology. Even then, is this enough? Considering a neurodegenerative disease such as Alzheimer's disease, for example, it would seem — if one considers the pre-clinical data — that we have generated effective treatments. Yet almost no clinical efficacy has been demonstrated in human populations. Why is it that we keep on attempting to use very similar mechanistic approaches, within very similar clinical trials, when there is almost no signal of efficacy (Espay & Stecher, 2020)? If one considers how approaches in the treatment of breast cancer changed very rapidly on the turn of a single clinical trial (Veronesi et al., 1981), where no differences in survival between radical mastectomy versus breast-conserving surgery was demonstrated (Veronesi et al., 2002), scientific communities have been able to change their approach, and paradigms, relatively quickly. These clinical contexts differ substantially, evidently, but the fundamental principle is clear.

Confounding short term observations with basic science and subsequently translatable science, at every step, must — I believe — be clouding our ability to resolve truth about biology from our models. Given that in our laboratories, the success or failure of experiments is very often seen through a lens of whether the data support the hypothesis and conclusions of some potential future manuscript, we set ourselves up to be victims of our scientific hubris (Diamandis & Bouras, 2018). My hope with the data generated in this PhD we minimise this error, perhaps even at the cost of extrapolating.

In PD research (as in many diseases), we clearly distinguish between disease and *health*. This is, of course, a model. It is useful and necessary but taking the binary distinction for granted, without pause, is likely clouding our ability to understand PD. Especially if one considers that this binary will impact the grouping of individuals who are included in a GWA study, the fibroblasts that will be re-programmed into neurons to study cell biology, the cells that are counted in post-mortem tissues, and the patients that will or not be included in a clinical trial.

We often hear the over-used adage, "all models are wrong, but some are useful" (attributed to George Box ("All Models Are Wrong," 2021)). However, I believe it germane to follow this quote with the following "a theory has only the alternative of being right or wrong. A model has a third possibility: it may be right, but irrelevant." (Eigen, 1973). Models are helpful; there is no doubt. But without robust theory, which informs our models, and importantly, keeps us mindful of the fact that our models are models, after all, it will be challenging to discover what we need to discover.

15.02 Considerations on selective vulnerability

Why are certain neurons vulnerable in PD, and others are not? I suggest, therefore, that to answer this question, one needs an acute understanding of the layered models which are applied in formulating this question (Table 7).

Binary distinctions made (models)			Notes and questions
Healthy (ageing)	vs	Parkinson disease	What is *healthy* ageing anyway? *PD*, is this a spectrum, and/or Parkinson diseases (PDs) — plural?
Physiology	vs	Pathophysiology	In certain PDs, this may be pathophysiology (i.e. SNCA expansion); in others, it is possible that this is only a form of physiological ageing?
Humans	vs	Animals	Animal models of "PD" capture what, exactly, about human PD? Do animals have the biology to 'get' PD?
Non-vulnerable neurons	vs	Vulnerable neurons	What about non-neuronal cells? Which neurons, specifically? In what order are they affected — is this important?

Table 7 Models and questions in PD research

The notion that certain neurons dysfunction and degenerate, whilst others do not, is evident — for the sake of simplicity *PD vs non-PD neurons.* This dysfunction and degeneration of specific circuits has been elucidated by both the clinical symptoms and *in vivo* imaging, as well as by the neuropathological evidence. This, however, has its caveats (as outlined in chapters 2 and 3). The way these cells have been counted warrants re-addressing, and 'what', in terms of specific cell-type, has also been counted, is not clear. Therefore, the sub-set, neurochemically speaking, of PD-neurons that are vulnerable is not exactly clear (except for perhaps SNc DA neurons and TH+ LC neurons). If we are to understand the vulnerability as a function of the morphology and physiology and try to understand how pathophysiology may be selectively affecting these PD-vulnerable neurons, we need to be prepared that the *PD vs non-PD neuron* is perhaps not accurate and that we are perhaps grouping neurons incorrectly (or not even considering other types). For example, the SNc/VTA dichotomy is pervasive in our literature (and I recognised the irony given the data and dichotomisation of the SNc/VTA in Chapter 4). However, the claim that the VTA is not vulnerable in PD is overtly false. It is likely less vulnerable than the SNc, with estimates across all studies suggesting that decreases in the SNc (DA neurons) numbers compared to controls of 67% and 53% for the VTA (Alberico et al., 2015).

Again, there is very limited actual data on the temporal order of this loss. As we showed in Chapter 2, there is very limited quantification of neuron numbers in multiple anatomically defined regions within the same cases and very limited data on clinical variables such as progression or disease duration. In some of the field's best work, Huynh et al., have modelled the temporal order of SNc, VTA, and LC (TH+) cell loss (referred to in chapter 3, letter to the editor) — shown in Figure 37 (Huynh et al., 2021). We can see that, according to this modelling (and one could dispute the choice of modelling) that at the time of

diagnosis, there is already substantial loss of SNc DA neurons but no VTA DA loss. However, these models are drawn from a relatively small data set (less than n=10 for each group), making this conclusion only tentative. Furthermore, given that other quantifications of VTA DA neurons show a loss at between 0–5 years of disease duration, it seems improbable that there is no VTA DA neuron loss at the time of diagnosis (as per Figure 37).

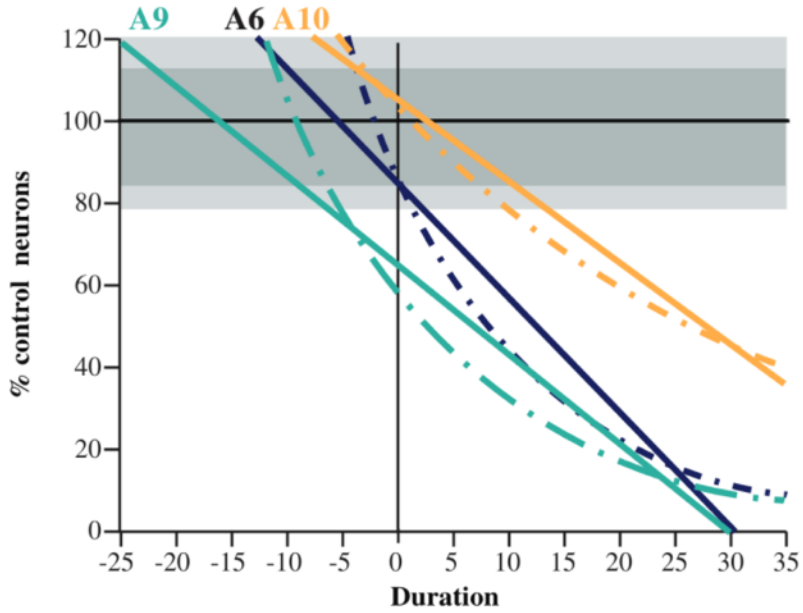


Figure 37 Regression modelling of cell loss as a function of disease duration for A9 (SNc), A10 (VTA), and A6 (LC) neurons

Overlaid graphs of the regression modelling with curve fitting comparing the decline in the A6 noradrenergic and A9 and A10 dopaminergic neurons (Huynh et al., 2021)

The inspiration for Chapter 2, the review on the cell loss literature (Giguère et al., 2018), was born out of an observation that when one was to seek the primary data supporting claims of cell loss, these were not forthcoming with the rabbit hole of citations. And Chapter 3, letter to the editor, was about the nature of claims citing this review, all through the lens of understanding how notions of PD-vulnerable neurons exists within our literature. Robust scientific writing is exceptionally challenging. And an obvious possibility is that some authors fail to read thoroughly enough the work they choose to cite (and I must confess, despite my initial naïve and hopeful intentions, many of the claims made in this thesis are not citing literature in a manner that would satisfy these initial intentions, fully).

It is widely recognised (one would hope) that scientific citation practices are opaque, and that scientists-in-training receive insufficient guidance on citing (Jamieson, 2019) — even clear expectations and the purpose of a citation are unclear, even at the level of publication and more 'official' channels of scientific communication. Some descriptions of how to cite have been published in readily digestible formats (Penders, 2018), and machine-readable citing systems have been developed (Shotton, 2010) that hope to add an extra layer of annotation to the purpose of the citation. The uptake of these practices, however, appears slow and disjointed, at best. Furthermore, the obscure role citations now play in academia have been depicted elegantly (Rekdal, 2014). And, interestingly, mathematical modelling purports that perhaps only 20% of citers read the source they cite (Simkin & Roychowdhury, 2002). However, in Chapter 3, we did find some clear examples (of the seven) claims citing the core message of our review, including:

> "However, the magnitude and timing of this degeneration is poorly defined because of the constraints of working with post-mortem human tissue." (Gonzalez-Rodriguez et al., 2020); "Overall, clearly, there is cell loss in brain areas in addition to the SNc, but neuron loss in these other brain areas requires further validation …" (Gcwensa et al., 2021); "However, it should be noted that few studies have performed stereological quantification of neurons in the VTA and the one study to do so reported a loss of neurons in this dopaminergic regions." (Hijaz & Volpicelli-Daley, 2020).

These select examples perhaps give us hope that this almost dogmatic idea of which specific neurons degenerate in PD are changing and that novel work will emerge attempting to answer these questions. When we explore the physiology and morphology of PD vs non-PD neurons, much of this work is carried out in rodent models. Rodents, and mice, are great models for human biology for a plethora of reasons. But are they good models for the physiology and morphology of PD-vulnerable neurons? For all PD-vulnerable neurons vs non-vulnerable neurons? Is the pathophysiology that we hope to uncover, is it pertinently captured in the biology of rodents? Overt differences between projecting and non-projecting neurons, in terms axonal size and complexity, as well as several physiological variables, are obviously present in many of our mammalian models — but may begin to become less overt in invertebrate models.

In Chapter 4, we saw the selective vulnerability of PD-vulnerable neurons modelled — somewhat well — *in vitro*, using very young murine primary neurons. These data, at minimum support hypotheses of selective vulnerability. The differential vulnerability to oxidative stress induced by treatment with hydrogen peroxide suggests that there truly is an autonomous vulnerability that is different between these different neuronal populations. One could ask, is this because the theory is correct? Or instead, the model "may be right, but irrelevant"; that it is to say, in this system, the data support the hypothesis that PD-vulnerable neurons are selectively vulnerable to elevated oxidative stress, even *in vitro*. Despite an *in vitro* system

being unable to capture major elements of what we are trying to model: an aged neuron (~60 years), within the full complement of brain tissues, with axonal arborisations totalling at least, at minimum, more than ~5 meters: is it able to capture the core biology that renders neurons vulnerable to PD? If so, why don't mice 'get' PD?

Do other animals get PD or other neurodegenerative diseases? In much of the literature, it is often claimed that neurodegenerative diseases are an exclusively human phenomena, and possible explanations for this have been suggested. As cited in "The Evolution-Driven Signature of Parkinson's Disease" (Diederich et al., 2020), which will be discussed, these ideas can be drawn back to fundamental work by Parent and colleagues where it was stated that "structures that appear early in the evolution are among the first to undergo involution in ageing diseases" and "the neurodegenerative processes at play specifically target the most phylogenetically ancient components of the brain, including the SN" (Parent, 1997). (Note: It is worth considering that some of the evidence, which is used to form these ideas within his article "The brain in evolution and involution", especially in the dichotomisation of SNc versus VTA differential vulnerability, is supported by data from MPTP treatment in primates, rather than post-mortem tissue in cases of PD).

In an elegant brief communication, Paul Manger says, "The majority of neuroscience research is focused on 3 species, rat, mouse and human. The general aim is to understand the basis of human neural function and dysfunction, and to develop therapeutics or interventions; however, over 80% of potential therapeutics developed in rodent models do not translate to the human condition, i.e. these rodent models are inefficient" (P. Manger, 2019) (P. Manger, 2019). Of course, these repeated failures are perhaps merely a reflection of a fact that our knowledge of biology is far from complete, and that our attempts to test potential therapies are often too late in disease progression.

Though limited, there is a neuroscience literature in a greater diversity of species. This is useful because many of the functional and structural systems are well conserved across vertebrates, and especially mammals (Cisek, 2021; P. R. Manger, 2020; Yamamoto & Vernier, 2011). Are there examples of human (brain) diseases in other species?

Age is the greatest risk factor for cognitive decline and many neurodegenerative diseases; therefore, it is an essential variable to consider when seeking to discover human, age-associated diseases, in the wild. Mild cognitive impairment often precedes a diagnosis of dementia in humans. Interestingly, cognitive impairment has been described in one-third of dogs by age 12 and two-thirds by age 16. Furthermore, dyskinesias in dogs have been reported purportedly due to basal ganglia dysfunction. Additionally, *Alzheimer's-disease-related-pathology* (Aβ plaques, neurofibrillary-related changes and cognitive deficits) have been reported in dogs, cats, dolphins, sheep, goats, wolves, polar bears, lemurs, chimpanzees, rhesus

monkeys, African green monkeys, and squirrel monkeys (Devinsky et al., 2018). Interestingly, rather obscure but fascinating hypotheses have been put forward to suggest that post-reproductive life span may be a key variable in terms of age and occurrence of Alzheimer's. Gunn-Moore and colleagues put forward the idea that the known link between failure in insulin signalling, type-2 diabetes, and Alzheimer's may be a consequence of a long post-reproductive life span. Dolphins are animals with long post-reproductive life spans, and both tangle and plaque (Alzheimer's) pathology have been observed in stranded animals (Gunn-Moore et al., 2018; Sarasa & Gallego, 2006). "Pathology-like", however, is of course not human disease.

Longevity, or rather, elevated mean age — at least in the context of a post-industrialised society — seems to be uniquely human. However, data does exist — weak in nature, but existent nonetheless — showing significant longevity "in the wild". Elephants are reported to live well up to 70–80 years (Chusyd et al., 2021), and Bowhead whales (Balaena mysticetus), for example, have been estimated to live well over 100 years consistently. Evidence even suggests that there have been individuals over 200 years old (George et al., 1999; Keane et al., 2015). Other work exists showing "AD pathology" as well as α-synuclein Lewy pathology in nine species of stranded toothed whales. The authors note, however, that they interpret these data as being potentially due to hypoxia (rather than ageing) due to the diving nature of the animals — positing the question as to which is causative (Sacchini et al., 2020). Notwithstanding, there does seem to be an absence of evidence for overt neurodegenerative diseases in the wild, rather than evidence of absence.

The core ideas that summarise why PD may be an exclusively human disease are best described in Garcia-Ruiz & Espay, 2017, Diederich et al., 2020, and Diederich et al., 2019. These ideas are elegant and well-substantiated by the data. Some of these ideas can trace their origins back to Parent's work, suggesting that the most evolutionary conserved components of the basal ganglia — which seem to have diverged ~500 million years ago — remain relatively similar across the taxa [Cyclostomes (lamprey), Chondrichthyes (sharks), Osteichthyes (lungfish), and Tetrapods (amphibians, reptiles, birds, and mammals)], and are the most vulnerable in disorders of ageing, in particular PD (Parent, 1997). Why then might SNc DA neurons in humans be vulnerable to PD, but those in the rodent, not?

Despite such conserved network structure and physiology, mammalian brain evolution has generated great diversity. Ancestral mammalian brains were relatively small but have evolved to be highly variable in size: modern mammalian brains vary in size by 100,000-fold (Count, 1947). However, this crude measurement does little to interrogate the variation in the composition of the brain. It is suggested that the variability in mammalian brains is due to varying mechanisms of scaling. For example, the expansions of the cerebral cortex across species are not consistent with increases in brain size (Herculano-Houzel, Manger, et al., 2014). The African elephant brain is ~3 times larger than the human brain, containing ~257 billion neurons. However, ~98% of these neurons are found in an enlarged cerebellum. In contrast, their

cerebral cortex has two times the mass of that found in humans, with only 5.6 billion neurons: roughly one-third of those found in humans (Herculano-Houzel, Avelino-de-Souza, et al., 2014) (though unintuitive, this is not an error). The mechanisms of scaling involve both the expansion (or reduction) of neuron numbers within specific structures, but also the variation in the size of these neurons. The human brain is, in fact, somewhat larger than would be expected based on body mass when compared to other primates (Figure 38).

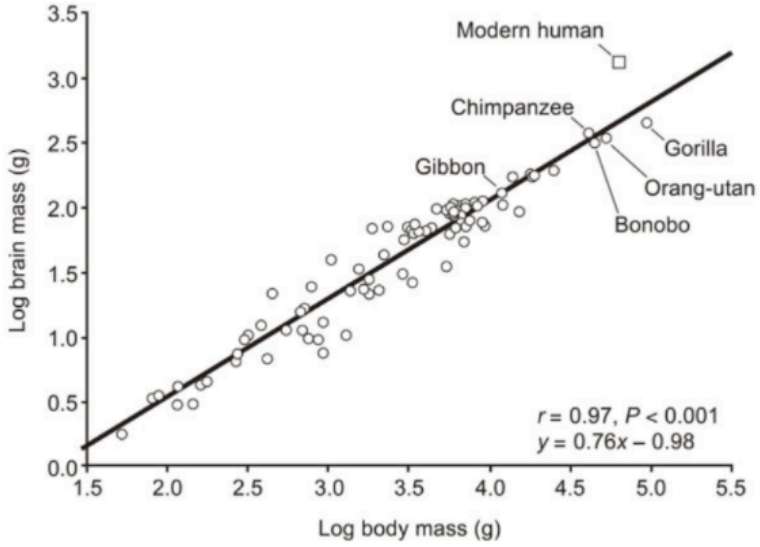


Figure 38 Brain mass versus body mass of primates

(Diederich et al., 2019)

However, what may truly differentiate the human brain is the ratio of scaling of certain structures within the basal ganglia. The elegant studies described previously (Figure 15) by Matsuda showed how a single SNc DA neuron occupied 6% of the volume of the striatum in rats. The volume of the striatum, however, in humans, has expanded to 300 times that of the rat. The number of neurons, however, has only increased by 32-fold (from 12,000 in rats to ~300,000–400,000 in humans). This expansion results in dopamine neurons within relatively similar structures and circuits having to innervate around ten times the functional domain (Diederich et al., 2019; Garcia-Ruiz & Espay, 2017). This expansion is summarised in Figure 39.

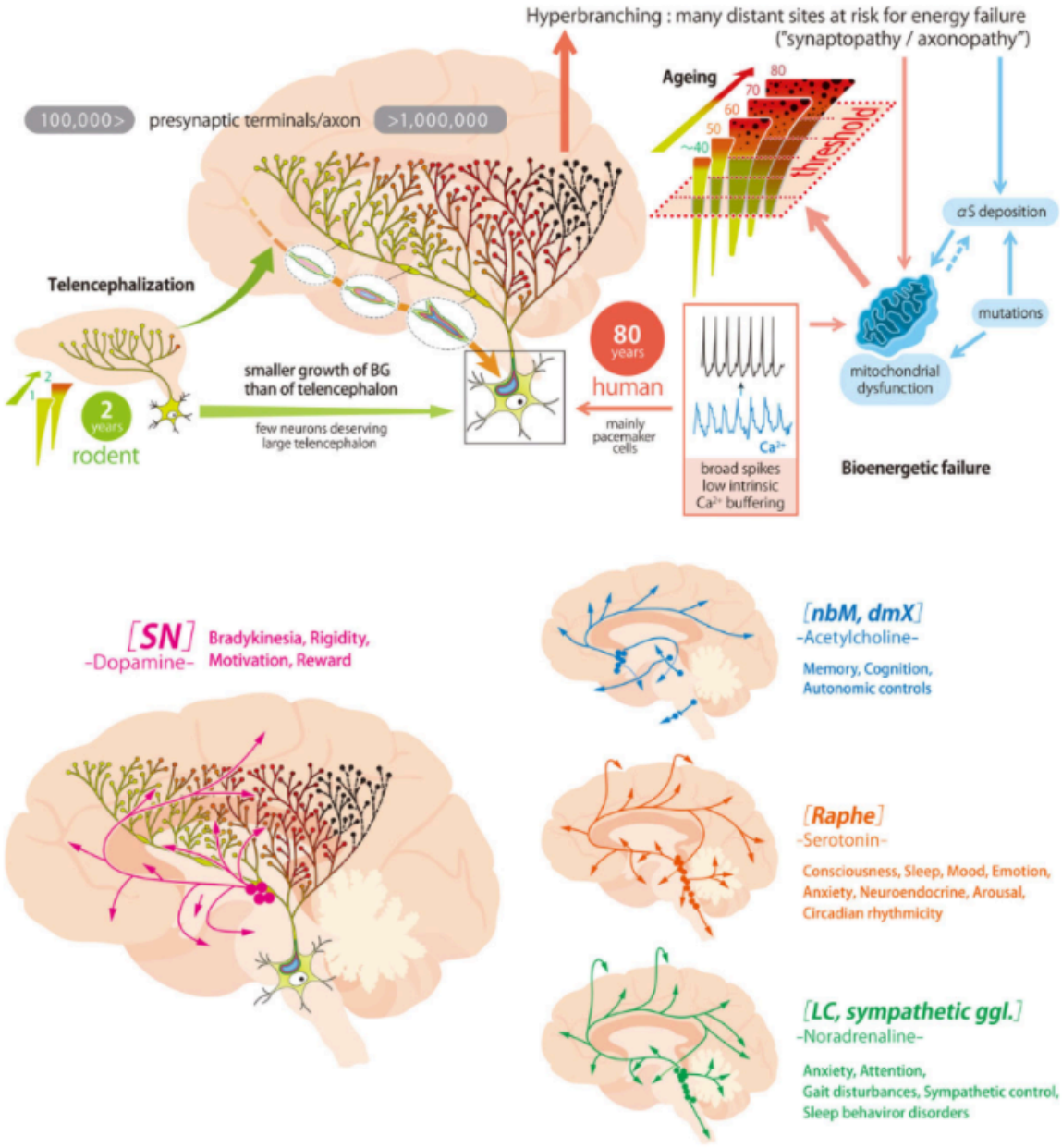


Figure 39 Schematic diagram depicting the impact of telencephalisation.

The disproportionately increasing the ratio of functional domain volume to the number of neurons of evolutionarily conserved components of the basal ganglia — principally SNc DA neurons. It is thought that this expansion renders these cells vulnerable to the insults associated with the PD. Furthermore, it is hypothesized that the other principle neuromodulator systems have been equally "left behind", evolutionary speaking (Diederich et al., 2019).

This could mean that a single SNc DA neuron, which has to remain functional for a lifetime, has likely over 1 million pre-synaptic terminals along an axonal domain totalling around 4.5 meters (as estimated by Bolam & Pissadaki, 2012). However, given estimated total lengths for other neuromodulatory

projecting neurons (described in Chapter 1) being an order of magnitude larger (Wu et al., 2014) (these claims, however, warrant further validation) than SNc DA neurons, it seems paramount to validate whether these neurons are equally, or even more vulnerable than SNc DA neurons.

15.03 Further interpretation of experimental data

Interestingly, as we see in Chapter 4, even *in vitro,* we see these projecting neurons growing highly branched and very long axonal domains that seem to correlate with relative vulnerability to oxidative stress induced by hydrogen peroxide. The subtle yet interesting observation is that what may differentiate even the projecting neurons is the number of mature pre-synaptic terminals We do not have data to know whether this phenomenon is an artefact of the model system or whether the activity and density of neurotransmitter release sites in the axonal domain of these purported PD-vulnerable projecting neurons is actually the key variable rendering them vulnerable in PD, in humans. In Chapter 4, we discussed possible interpretations of our data, specifically the absence of overt differences in ROS production: despite other functional and morphological differences. It is of course possible that roGFP is not a probe sensitive enough to distinguish small variabilities in local ROS production relevant to homeostatic limits of the cell. Or that ROS production is distributed throughout the axonal domain, and we would have been better placed to attempt to measure ROS production using a probe localised to the cytoplasmic domain such as cyto-roGFP, rather than MTS-roGFP, targeted to mitochondria specifically. Another important factor we did not evaluate was the intrinsic firing rates of all our neurons in culture. It is highly possible that in our culture conditions, at 10 DIV, the majority of these neurons had not matured enough to begin to exhibit their defining pacemaker firing. It is also possible that *in vitro* many of these neurons do not have their specific connectivity which may be required to induce the developmental programs essential for a full physiological maturation.

Perhaps, if we assume the premise that we should observe differences, these data could be simply explained by the lack of appropriate axonal growth. Given estimates of axonal lengths *in vivo*, differences in ROS production may only arise when the axonal domain is at least orders of magnitude larger than those observed *in vitro* where our axonal arborization are around 20 times smaller than *in vivo.* In future work it would perhaps be fruitful to try to emulate *in vivo* size axonal arborizations. This would perhaps be possible by extending the time of culture — aiming to optimize conditions to achieve cultures that last months rather than weeks. Another potential approach would be to target genes that drive axonal growth, in an attempt to engineer even larger SNc DA neurons. Would this render the cell closer to an *in vivo-like* neuron who would be sensitive to the bioenergetic costs associated with such large axonal domains. Of course, this implies the assumption that an axonal domain within the size ranges of rodents is ‘vulnerable’ from a bioenergetic point of view. Data from our lab suggests that it is possible to increase the axonal arborization of single SNc DA neurons using multiple models (Tanguay et al., 2021), where we can increase their vulnerability to 6-OHDA

(but not, interestingly, α-synuclein) (Giguère et al., 2019). By increasing the ratio of axonal domain to single SNc DA neuron, would we be able to generate a more 'humanized' mouse model. Perhaps in this form the mouse may become spontaneously parkinsonian if coupled with accelerated ageing models, or the insertion of known genetic risk factors for PD.

There is not necessarily something in biology called PD, per se, but rather many biological pathways or (mal)adaptations that manifest in such a way that result in consistent clinical presentation (and pathophysiology) — thus the diagnosis. It can be presumed that the emergence of clinical phenotypes (which inform the diagnosis) arise due to the dysfunction of common systems — independent of the reason for dysfunction in itself. For example, whether it be an individual with genetically linked young-onset PD (Reed et al., 2019), or individuals who consumed recreational drugs containing the toxin MPTP (Langston, 2017), the dysfunction at the level of the brain system contributes to the clinical manifestation, independently of the cellular/biochemical pathways differentially affected (I appreciate that given modern diagnostic criteria that these individuals would be categorised differentially). It is, therefore, important to isolate the biological features (or physiology and function) that are vulnerable to the insults that generate the clinically categorised phenotype we call PD.

Throughout the literature and this thesis, we have seen a plethora of biological systems which may or may not be key causes of PD. Figure 40 for example, illustrates the concept that PD may be visualised as a puzzle of potential pathologies, all capable of causing "PD". Though not discussed in either publication, I believe it plausible that any and all of these systems may be capable of generating "PD". These biological systems are perhaps the most integral to the function of projecting neuromodulatory neurons, with a physiology associated with intense calcium management. Perhaps PD is defined by this puzzle, and ALS, or AD, is defined by a different puzzle (with substantial overlap).

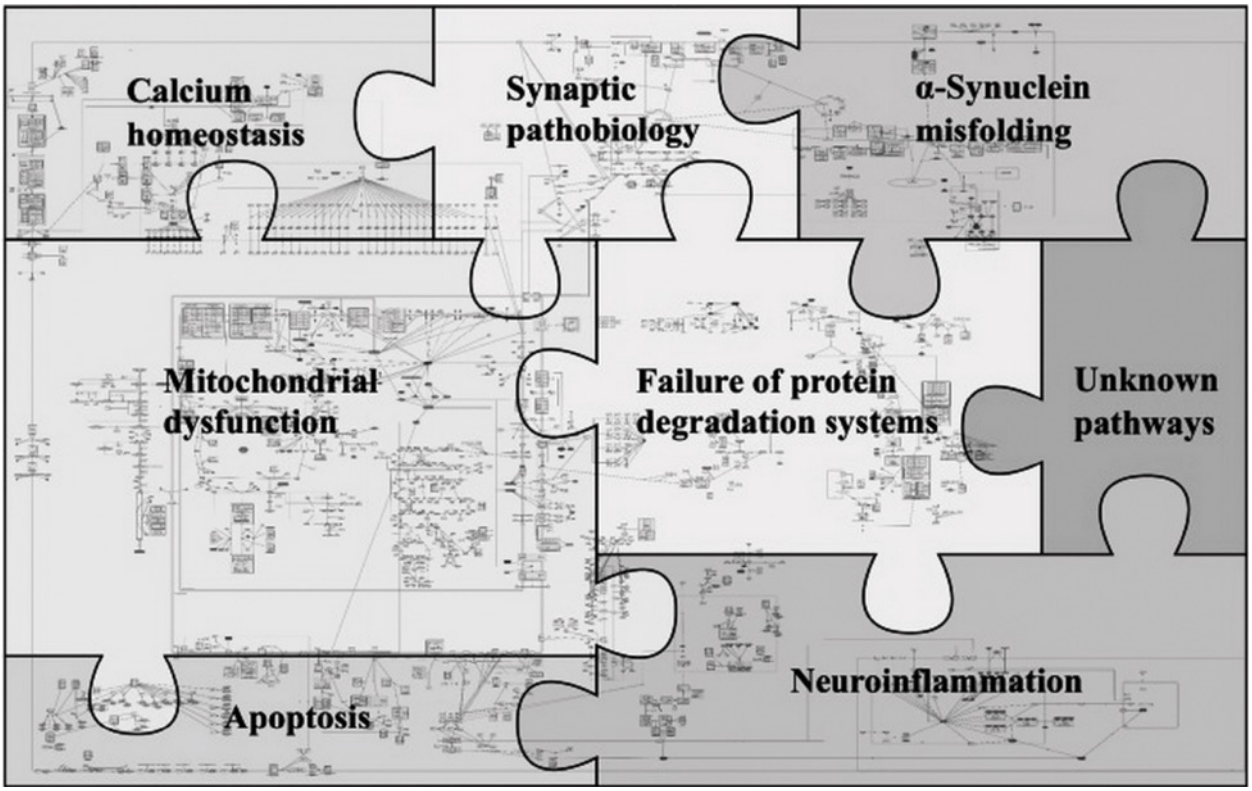


Figure 40 PD as a puzzle

Analytic methods by a Japanese team created a "map of molecular mechanisms and pathways considered to be the key players in the disease" based on 2,285 elements and 989 reactions supported by 429 articles and 254 entries from publicly available bioinformatic databases. The map shows the range of abnormalities ever described, including synaptic and mitochondrial dysfunction, failure of protein degradation systems, alpha-synuclein misfolding, and neuroinflammation (Espay & Stecher, 2020; Fujita et al., 2014).

How might we understand this in the context of all we know about the genetics of PD? I would propose that each of these systems can be dysfunctional, but it is only the critical mass of many of these dysfunctional systems that generates crossing the critical limit for physiology, or rather extra-homeostatic conditions. An SNCA triplication perhaps is a big enough "dose" of system dysfunction that it causes degeneration of "PD neurons". And a medium dose of mitochondrial dysfunction, and protein degradation dysfunction, causes "PD" neurons to degenerate. And of course, degeneration that is generated may only be observable in the context of a "PD-puzzle", in a human neuron. Perhaps a view like this explains, quite easily, why no two PDs are quite alike. They are similar enough to be categorised together, but not for the subjective experience for the patient to be the same. It would naïve, therefore, to aim to treat — with the aim of disease modification — all cases of PD with a PD-biological-subtype-specific target.

Finally, the distinction of PD vs healthy is fundamental (as is the distinction between disease and non-disease (Smith, 2002)). How clear is the distinction between ageing, and PD. Is it possible that this is a spectrum for at least certain forms of PD? One example of the importance of this question can be illustrated by the lack of solid information on neuron numbers. Both in healthy ageing, as well as in healthy vs disease. The biology of ageing is a vast field growing rapidly. However, we can make some astute observations on this fundamental variable, cell numbers. Though data is still somewhat weak, there is evidence to suggest that the number of SNc DA neurons declines with age, with the most robust estimates suggesting a decrease of roughly 10% per decade, on average. This rate, and how it changes during life (i.e., whether this number is stable until 50 years, and then declines rapidly) is unclear (Ma et al., 2003). Furthermore, there are tentative reports — and given their nature, requiring sensitivity — that decline in SNc DA neuron numbers vary by ethnic ancestry. One such report suggests that lower incidences of PD in individuals of Asian Indian ancestry (Ben-Joseph et al., 2020; Muthane et al., 2001) may be accounted for by differential loss of SNc DA neurons over time (Alladi et al., 2009) — with individuals of Asian Indian ancestry showing an "absence of age-related changes in nigral dopaminergic neurons". Of course, extraordinary claims require extraordinary evidence. But the suggestion that genetic variability may be available to understand differential vulnerability of SNc DA neurons to both age and disease is promising. The number of TH+ locus coeruleus neurons has also be quantified as a function of age, and data suggests that total numbers may halve over a 100-year life span (however, this is from a limited data set: 20 individuals, from 1–100 years of age) (Manaye et al., 1995). In addition, the severity of LC neuronal loss has been correlated with the incidence of dementia in PD (Zarow et al., 2003; Zweig et al., 1993). Given that we are beginning to appreciate that not all individuals age, biologically, at the same rate (Belsky et al., 2015; Santos et al., 2021), could PD arise because of an accelerated ageing phenotype ('ageotype' — (Ahadi et al., 2020)), rather than say a genetic or environmental risk profile causing selective degeneration of "PD-nuclei"?

15.04 Future directions

In the context of this discussion and thesis, LP has somewhat been neglected in attempting to understand the selective degeneration observed in PD. We have observed a restricted and staged LP in the work by Braak and colleagues (Braak & Del Tredici, 2017), the 'spreading' of LP in long-term fetal nigral transplants (Kordower & Brundin, 2009), and an abundant literature on possible toxic effects of 'pathological' α-synuclein. However, given that PD, or PD-like diseases, are reported without LP (Johansen et al., 2018; Takanashi et al., 2018), and that LP is present in normal elderly subjects (Knopman et al., 2003; Markesbery et al., 2009; Menšíková et al., 2022), it seems logical that the focus must remain on the neurons that are affected, and not the subsequent pathology that we observe. Furthermore, the clinical subtyping of

PD has been shown to be un-correlated to the neuropathological findings (De Pablo-Fernández et al., 2019; Espay & Marras, 2019).

To conclude, though we have may have some confidence in which neurons are degenerating in PD — given the fundamental nature of this knowledge to our endeavours to understand PD — novel thorough investigations asking which neurons and cells are affected in PD, as well as other neurodegenerative diseases, is essential. Secondly, I believe that our data showing that the vulnerability to cell stress may be conferred by the number of active neurotransmitter release sites in projecting neuromodulatory neurons, suggests that this unique characteristic may be a critical factor rendering neurons vulnerable to dysfunctional biological pathways (outlined in Figure 40) associated with PD.

The phenotyping of neuron sub-types that is currently being undertaken (such as the work at the Allen Institute (Abbott, 2021; Peng et al., 2021)) is currently somewhat limited to cortical neurons and must be continued into the neuronal types of interest for diseases such as PD — projecting neuromodulatory neurons. With the advent of novel methods, we may be in the position in the not-too-distant future to carry out brain-wide spatial transcriptomics (Marx, 2021) coupled with stereology-like automated quantifications — perhaps across neurodegenerative disease categories, and age — coupled with longitudinal, rich clinical, functional, and behavioural data to allow us to probe which neurons, precisely, and when, dysfunction beings to arise. At the time of writing, we currently have a subsequent meta-analysis being undertaken evaluating the evidence for cell loss in PD (Lunt et al., 2021). It is hoped that with these new analyses of existing data, coupled with our review (Giguère et al., 2018) on the cell loss data in PD, we will be able to encourage our peers that a thorough evaluation of specific cell loss, across multiple brain regions, within the same, well-documented cases of PD, is essential.

15.05 Closure

What a privilege it is to be a scientist. To discover for the sake of discovery itself; to improve the lives of people, present and future. I think a fitting observation to hold following a PhD is well described by the late S Gould:

> "But, as we consider the totality of similarly broad and fundamental aspects of life, we cannot defend division by two as a natural principle of objective order. Indeed, the 'stuff' of the universe often strikes our senses as complex and shaded continua, admittedly with faster and slower moments, and bigger and smaller steps, along the way. Nature does not dictate dualities, trinities, quarterings, or any 'objective' basis for human taxonomies; most of our chosen schemes, and our designated numbers of categories, record human choices from a

cornucopia of possibilities offered by natural variation from place to place, and permitted by the flexibility of our mental capacities. How many seasons (if we wish to divide by seasons at all) does a year contain? How many stages shall we recognise in a human life?"

— Stephen Jay Gould (2003)

16 Bibliography

Abbott, A. (2021). How the world's biggest brain maps could transform neuroscience. *Nature*, *598*(7879), 22–25. https://doi.org/10/gm287n

Agnati, L. F., Zoli, M., Strömberg, I., & Fuxe, K. (1995). Intercellular communication in the brain: Wiring versus volume transmission. *Neuroscience*, *69*(3), 711–726. https://doi.org/10.1016/0306-4522(95)00308-6

Ahadi, S., Zhou, W., Schüssler-Fiorenza Rose, S. M., Sailani, M. R., Contrepois, K., Avina, M., Ashland, M., Brunet, A., & Snyder, M. (2020). Personal aging markers and ageotypes revealed by deep longitudinal profiling. *Nature Medicine*, *26*(1), 83–90. https://doi.org/10.1038/s41591-019-0719-5

Anden N-E, Fuxe K, Hamberger B, Hokfelt T. 1966. A quantitative study on the nigro-striatal dopamine neuron system in the rat. Acta Physiologica Scandinavica 67:306–312.

Alberico, S. L., Cassell, M. D., & Narayanan, N. S. (2015). The Vulnerable Ventral Tegmental Area in Parkinson's Disease. *Basal Ganglia*, *5*(2–3), 51–55. https://doi.org/10.1016/j.baga.2015.06.001

All models are wrong. (2021). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=All_models_are_wrong&oldid=1046298382

Alladi, P. A., Mahadevan, A., Yasha, T. C., Raju, T. R., Shankar, S. K., & Muthane, U. (2009). Absence of age-related changes in nigral dopaminergic neurons of Asian Indians: Relevance to lower incidence of Parkinson's disease. *Neuroscience*, *159*(1), 236–245. https://doi.org/10.1016/j.neuroscience.2008.11.051

Armstrong, M. J., & Okun, M. S. (2020a). Diagnosis and Treatment of Parkinson Disease: A Review. *JAMA*, *323*(6), 548–560. https://doi.org/10.1001/jama.2019.22360

Armstrong, M. J., & Okun, M. S. (2020b). Time for a New Image of Parkinson Disease. *JAMA Neurology*, *77*(11), 1345–1346. https://doi.org/10.1001/jamaneurol.2020.2412

Avery, M. C., & Krichmar, J. L. (2017). Neuromodulatory Systems and Their Interactions: A Review of Models, Theories, and Experiments. *Frontiers in Neural Circuits*, *11*, 108. https://doi.org/10.3389/fncir.2017.00108

Baker KG, Halliday GM, Hornung JP, Geffen LB, Cotton RG, Törk I. 1991. Distribution, morphology and number of monoamine-synthesizing and substance P-containing neurons in the human dorsal raphe nucleus. Neuroscience 42:757–775. doi: 10.1016/0306-4522(91)90043-N.

Badanjak, K., Fixemer, S., Smajić, S., Skupin, A., & Grünewald, A. (2021). The Contribution of Microglia to Neuroinflammation in Parkinson's Disease. *International Journal of Molecular Sciences*, *22*(9), 4676. https://doi.org/10/gm5zwr

Bandres-Ciga, S., Diez-Fairen, M., Kim, J. J., & Singleton, A. B. (2020). Genetics of Parkinson's disease: An introspection of its journey towards precision medicine. *Neurobiology of Disease*, *137*, 104782. https://doi.org/10/gjgjtj

Banerjee, A., Lee, J., Nemcova, P., Liu, C., & Kaeser, P. S. (2020). Synaptotagmin-1 is the Ca2+ sensor for fast striatal dopamine release. *ELife*, *9*. https://doi.org/10.7554/eLife.58359

Barbeau, A. (1969). L-Dopa Therapy in Parkinson's Disease. *Canadian Medical Association Journal*, *101*(13), 59–68.

Bates, G. P., Dorsey, R., Gusella, J. F., Hayden, M. R., Kay, C., Leavitt, B. R., Nance, M., Ross, C. A., Scahill, R. I., Wetzel, R., Wild, E. J., & Tabrizi, S. J. (2015). Huntington disease. *Nature Reviews. Disease Primers*, *1*, 15005. https://doi.org/10.1038/nrdp.2015.5

Belsky, D. W., Caspi, A., Houts, R., Cohen, H. J., Corcoran, D. L., Danese, A., Harrington, H., Israel, S., Levine, M. E., Schaefer, J. D., Sugden, K., Williams, B., Yashin, A. I., Poulton, R., & Moffitt, T. E. (2015). Quantification of biological aging in young adults. *Proceedings of the National Academy of Sciences*, *112*(30), E4104–E4110. https://doi.org/10.1073/pnas.1506264112

Ben-Joseph, A., Marshall, C. R., Lees, A. J., & Noyce, A. J. (2020). Ethnic Variation in the Manifestation of Parkinson's Disease: A Narrative Review. *Journal of Parkinson's Disease*, *10*(1), 31–45. https://doi.org/10.3233/JPD-191763

Berg, D., Borghammer, P., Fereshtehnejad, S.-M., Heinzel, S., Horsager, J., Schaeffer, E., & Postuma, R. B. (2021). Prodromal Parkinson disease subtypes—Key to understanding heterogeneity. *Nature Reviews Neurology*, *17*(6), 349–361. https://doi.org/10.1038/s41582-021-00486-9

Birkmayer, W., & Hornykiewicz, O. (1961). [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. *Wiener Klinische Wochenschrift*, *73*, 787–788.

Blanchet, P. J., & Brefel-Courbon, C. (2018). Chronic pain and pain processing in Parkinson's disease. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *87*(Pt B), 200–206. https://doi.org/10.1016/j.pnpbp.2017.10.010

Boka, G., Anglade, P., Wallach, D., Javoy-Agid, F., Agid, Y., & Hirsch, E. C. (1994). Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neuroscience Letters*, *172*(1), 151–154. https://doi.org/10.1016/0304-3940(94)90684-X

Bolam, J. P., & Pissadaki, E. K. (2012). Living on the edge with too many mouths to feed: Why dopamine neurons die. *Movement Disorders*, *27*(12), 1478–1483. https://doi.org/10.1002/mds.25135

Boncristiano S, Calhoun ME, Kelly PH, Pfeifer M, Bondolfi L, Stalder M, Phinney AL, Abramowski D, Sturchler-Pierrat C, Enz A, Sommer B, Staufenbiel M, Jucker M. 2002. Cholinergic changes in the APP23 transgenic mouse model of cerebral amyloidosis. The Journal of Neuroscience 22:3234–3243.

Borghammer, P., & Van Den Berge, N. (2019). Brain-First versus Gut-First Parkinson's Disease: A Hypothesis. *Journal of Parkinson's Disease*, *9*(s2), S281–S295. https://doi.org/10.3233/JPD-191721

Braak, H., & Del Tredici, K. (2017). Neuropathological Staging of Brain Pathology in Sporadic Parkinson's disease: Separating the Wheat from the Chaff. *Journal of Parkinson's Disease*, *7*(s1), S71–S85. https://doi.org/10.3233/JPD-179001

Braak, H., Del Tredici, K., Rüb, U., De Vos, R. A., Steur, E. N. J., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*(2), 197–211.

Brain health. (2021). WHO. https://www.who.int/westernpacific/health-topics/brain-health

Brás, J., Guerreiro, R., & Hardy, J. (2015). SnapShot: Genetics of Parkinson's Disease. *Cell*, *160*(3), 570-570.e1. https://doi.org/10.1016/j.cell.2015.01.019

Brooks, D. J. (2000). Dopamine agonists: Their role in the treatment of Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, *68*(6), 685–689. https://doi.org/10.1136/jnnp.68.6.685

Brudek, T., Winge, K., Folke, J., Christensen, S., Fog, K., Pakkenberg, B., & Pedersen, L. Ø. (2017). Autoimmune antibody decline in Parkinson's disease and Multiple System Atrophy; a step

towards immunotherapeutic strategies. *Molecular Neurodegeneration*, *12*(1), 44. https://doi.org/10.1186/s13024-017-0187-7

Bryois, J., Skene, N. G., Hansen, T. F., Kogelman, L. J. A., Watson, H. J., Liu, Z., Brueggeman, L., Breen, G., Bulik, C. M., Arenas, E., Hjerling-Leffler, J., & Sullivan, P. F. (2020). Genetic identification of cell types underlying brain complex traits yields insights into the etiology of Parkinson's disease. *Nature Genetics*, *52*(5), 482–493. https://doi.org/10/gg7kzz

Bucher, D., & Marder, E. (2013). SnapShot: Neuromodulation. *Cell*, *155*(2), 482-482.e1. https://doi.org/10.1016/j.cell.2013.09.047

Calabresi, P., Picconi, B., Tozzi, A., Ghiglieri, V., & Di Filippo, M. (2014). Direct and indirect pathways of basal ganglia: A critical reappraisal. *Nature Neuroscience*, *17*(8), 1022–1030. https://doi.org/10.1038/nn.3743

Callaway, E. M., Dong, H.-W., Ecker, J. R., Hawrylycz, M. J., Huang, Z. J., Lein, E. S., Ngai, J., Osten, P., Ren, B., Tolias, A. S., White, O., Zeng, H., Zhuang, X., Ascoli, G. A., Behrens, M. M., Chun, J., Feng, G., Gee, J. C., Ghosh, S. S., … Project management. (2021). A multimodal cell census and atlas of the mammalian primary motor cortex. *Nature*, *598*(7879), 86–102. https://doi.org/10/gm29p9

Canavier, C. C., Evans, R. C., Oster, A. M., Pissadaki, E. K., Drion, G., Kuznetsov, A. S., & Gutkin, B. S. (2016). Implications of cellular models of dopamine neurons for disease. *Journal of Neurophysiology*, *116*(6), 2815–2830. https://doi.org/10.1152/jn.00530.2016

Carlsson, A., Kehr, W., Lindqvist, M., Magnusson, T., & Atack, C. V. (1972). Regulation of monoamine metabolism in the central nervous system. *Pharmacological Reviews*, *24*(2), 371–384.

Chandler, D. J., Jensen, P., McCall, J. G., Pickering, A. E., Schwarz, L. A., & Totah, N. K. (2019). Redefining Noradrenergic Neuromodulation of Behavior: Impacts of a Modular Locus Coeruleus Architecture. *Journal of Neuroscience*, *39*(42), 8239–8249. https://doi.org/10/ggkd67

Chusyd, D. E., Ackermans, N. L., Austad, S. N., Hof, P. R., Mielke, M. M., Sherwood, C. C., & Allison, D. B. (2021). Aging: What We Can Learn From Elephants. *Frontiers in Aging*, *2*, 36. https://doi.org/10/gmvwwh

Cisek, P. (2021). Evolution of behavioural control from chordates to primates. *Phil. Trans. R. Soc. B.*, 31. https://doi.org/10.1098/rstb.2020.0522

Connolly, B. S., & Lang, A. E. (2014). Pharmacological Treatment of Parkinson Disease: A Review. *JAMA*, *311*(16), 1670–1683. https://doi.org/10.1001/jama.2014.3654

Couch, Y., Alvarez-Erviti, L., Sibson, N. R., Wood, M. J. A., & Anthony, D. C. (2011). The acute inflammatory response to intranigral α-synuclein differs significantly from intranigral lipopolysaccharide and is exacerbated by peripheral inflammation. *Journal of Neuroinflammation*, *8*, 166. https://doi.org/10.1186/1742-2094-8-166

Count, E. W. (1947). Brain and Body Weight in Man: Their Antecedents in Growth and Evolution. Annals of the New York academy of sciences.

Cunningham MG, Connor CM, Zhang K, Benes FM. 2005. Diminished serotonergic innervation of adult medial prefrontal cortex after 6-OHDA lesions in the newborn rat. *Brain Research Developmental Brain Research* 157:124–131. doi: 10.1016/j.devbrainres.2005.02.020.

Curiosity Creates Cures: The Value and Impact of Basic Research. (n.d.). Retrieved October 29, 2021, from https://www.nigms.nih.gov/education/fact-sheets/Pages/curiosity-creates-cures.aspx

Dauvilliers, Y. (2018). *REM sleep behaviour disorder*. 16.

Descarries, L., & Mechawar, N. (2000). Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. *Progress in Brain Research*, *125*, 27–47. https://doi.org/10.1016/S0079-6123(00)25005-X

Descarries L, Watkins KC, Garcia S, Beaudet A. 1982. The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study. The Journal of Comparative Neurology 207:239–254. doi: 10.1002/cne.902070305.

Devinsky, O., Boesch, J. M., Cerda-Gonzalez, S., Coffey, B., Davis, K., Friedman, D., Hainline, B., Houpt, K., Lieberman, D., Perry, P., Prüss, H., Samuels, M. A., Small, G. W., Volk, H., Summerfield, A., Vite, C., Wisniewski, T., & Natterson-Horowitz, B. (2018). A cross-species approach to disorders affecting brain and behaviour. *Nature Reviews Neurology*, *14*(11), 677–686. https://doi.org/10/gfk6sp

Diamandis, E. P., & Bouras, N. (2018). Hubris and Sciences. *F1000Research*, *7*, 133. https://doi.org/10.12688/f1000research.13848.1

Diederich, N. J., Surmeier, D. J., Uchihara, T., Grillner, S., & Goetz, C. G. (2019). Parkinson's disease: Is it a consequence of human brain evolution? *Movement Disorders*, *34*(4), 453–459. https://doi.org/10.1002/mds.27628

Diederich, N. J., Uchihara, T., Grillner, S., & Goetz, C. G. (2020). The Evolution-Driven Signature of Parkinson's Disease. *Trends in Neurosciences*, *43*(7), 475–492. https://doi.org/10.1016/j.tins.2020.05.001

Dismukes, R. (1979). *New concepts of molecular communication among neurons*. https://doi.org/10.1017/S0140525X00063317

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., Marshall, F. J., Ravina, B. M., Schifitto, G., Siderowf, A., & Tanner, C. M. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, *68*(5), 384–386. https://doi.org/10/cvt6bf

Dorsey, E. R., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J. C., Ansha, M. G., Brayne, C., Choi, J.-Y. J., Collado-Mateo, D., Dahodwala, N., Do, H. P., Edessa, D., Endres, M., Fereshtehnejad, S.-M., Foreman, K. J., Gankpe, F. G., Gupta, R., Hankey, G. J., … Murray, C. J. L. (2018). Global, regional, and national burden of Parkinson's disease, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, *17*(11), 939–953. https://doi.org/10.1016/S1474-4422(18)30295-3

Ducrot, C., Bourque, M.-J., Delmas, C. V., Racine, A.-S., Guadarrama Bello, D., Delignat-Lavaud, B., Domenic Lycas, M., Fallon, A., Michaud-Tardif, C., Burke Nanni, S., & others. (2021). Dopaminergic neurons establish a distinctive axonal arbor with a majority of non-synaptic terminals. *The FASEB Journal*, *35*(8), e21791.

Dugger, B. N., & Dickson, D. W. (2017). Pathology of Neurodegenerative Diseases. *Cold Spring Harbor Perspectives in Biology*, *9*(7), a028035. https://doi.org/10/ggzgsk

Economo, M. N., Winnubst, J., Bas, E., Ferreira, T. A., & Chandrashekar, J. (2019). Single-neuron axonal reconstruction: The search for a wiring diagram of the brain. *Journal of Comparative Neurology*, *527*(13), 2190–2199. https://doi.org/10.1002/cne.24674

Eigen, M. (1973). The Origin of Biological Information. In J. Mehra (Ed.), *The Physicist's Conception of Nature* (p. 618). Springer Netherlands. https://doi.org/10.1007/978-94-010-2602-4_30

Elliott, M. L., Caspi, A., Houts, R. M., Ambler, A., Broadbent, J. M., Hancox, R. J., Harrington, H., Hogan, S., Keenan, R., Knodt, A., Leung, J. H., Melzer, T. R., Purdy, S. C., Ramrakha, S., Richmond-Rakerd, L. S., Righarts, A., Sugden, K., Thomson, W. M., Thorne, P. R., … Moffitt,

T. E. (2021). Disparities in the pace of biological aging among midlife adults of the same chronological age have implications for future frailty risk and policy. *Nature Aging*, *1*(3), 295–308. https://doi.org/10/gk58cf

Espay, A., & Stecher, B. (2020). *Brain Fables: The Hidden History of Neurodegenerative Diseases and a Blueprint to Conquer Them* (1st ed.). Cambridge University Press. https://doi.org/10.1017/9781108888202

Falk, T., Mai, D., Bensch, R., Çiçek, Ö., Abdulkadir, A., Marrakchi, Y., Böhm, A., Deubner, J., Jäckel, Z., Seiwald, K., Dovzhenko, A., Tietz, O., Dal Bosco, C., Walsh, S., Saltukoglu, D., Tay, T. L., Prinz, M., Palme, K., Simons, M., … Ronneberger, O. (2019). U-Net: Deep learning for cell counting, detection, and morphometry. *Nature Methods*, *16*(1), 67–70. https://doi.org/10.1038/s41592-018-0261-2

Fang, F. C., & Casadevall, A. (2010). Lost in Translation—Basic Science in the Era of Translational Research. *Infection and Immunity*, *78*(2), 563–566. https://doi.org/10.1128/IAI.01318-09

Ferguson, S. M. (2018). Axonal transport and maturation of lysosomes. *Current Opinion in Neurobiology*, *51*, 45–51. https://doi.org/10.1016/j.conb.2018.02.020

Filippi, M. (2018). *Multiple sclerosis*. 27. https://doi.org/10/gfwvh9

Forno, L. S. (1987). The Lewy body in Parkinson's disease. *Advances in Neurology*, *45*, 35–43.

Franco-Iborra, S., & Perier, C. (2015). Neurodegeneration: The Size Takes It All. *Current Biology*, *25*(18), R797–R800. https://doi.org/10.1016/j.cub.2015.07.062

Fu, H., Hardy, J., & Duff, K. E. (2018). Selective vulnerability in neurodegenerative diseases. *Nature Neuroscience*, *21*(10), 1350–1358. https://doi.org/10.1038/s41593-018-0221-2

Fujita, K. A., Ostaszewski, M., Matsuoka, Y., Ghosh, S., Glaab, E., Trefois, C., Crespo, I., Perumal, T. M., Jurkowski, W., Antony, P. M. A., Diederich, N., Buttini, M., Kodama, A., Satagopam, V. P., Eifes, S., Del Sol, A., Schneider, R., Kitano, H., & Balling, R. (2014). Integrating pathways of Parkinson's disease in a molecular interaction map. *Molecular Neurobiology*, *49*(1), 88–102. https://doi.org/10.1007/s12035-013-8489-4

Fuxe, K. (2000). Opening address. In *Progress in Brain Research* (Vol. 125, p. ix). Elsevier. https://doi.org/10.1016/S0079-6123(00)25001-2

Garcia-Ruiz, P. J., & Espay, A. J. (2017). Parkinson Disease: An Evolutionary Perspective. *Frontiers in Neurology*, *8*, 157. https://doi.org/10.3389/fneur.2017.00157

Gauthier, J., Parent, M., Lévesque, M., & Parent, A. (1999). The axonal arborization of single nigrostriatal neurons in rats. *Brain Research*, *834*(1–2), 228–232. https://doi.org/10.1016/s0006-8993(99)01573-5

Gcwensa, N. Z., Russell, D. L., Cowell, R. M., & Volpicelli-Daley, L. A. (2021). Molecular Mechanisms Underlying Synaptic and Axon Degeneration in Parkinson's Disease. *Frontiers in Cellular Neuroscience*, *15*, 44. https://doi.org/10.3389/fncel.2021.626128

Genome-Wide Association Studies (GWAS). (n.d.). Genome.Gov. Retrieved October 15, 2021, from https://www.genome.gov/genetics-glossary/Genome-Wide-Association-Studies

George, J., Bada, J., Zeh, J., Scott, L., Brown, S. E., O'hara, T., & Suydam, R. (1999). Age and growth estimates of bowhead whales (Balaena mysticetus) via aspartic acid racemization. *Canadian Journal of Zoology*. https://doi.org/10.1139/Z99-015

Giguère, N., Burke Nanni, S., & Trudeau, L.-E. (2018). On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease. *Frontiers in Neurology*, *9*, 455. https://doi.org/10.3389/fneur.2018.00455

Giguère, N., Delignat-Lavaud, B., Herborg, F., Voisin, A., Li, Y., Jacquemet, V., Anand-Srivastava, M., Gether, U., Giros, B., & Trudeau, L.-É. (2019). Increased vulnerability of nigral dopamine neurons after expansion of their axonal arborization size through D2 dopamine receptor conditional knockout. *PLOS Genetics*, *15*(8), e1008352. https://doi.org/10.1371/journal.pgen.1008352

Goedert, M., Spillantini, M. G., Del Tredici, K., & Braak, H. (2013). 100 years of Lewy pathology. *Nature Reviews Neurology*, *9*(1), 13–24. https://doi.org/10.1038/nrneurol.2012.242

Goetz, C. G. (2011). The History of Parkinson's Disease: Early Clinical Descriptions and Neurological Therapies. *Cold Spring Harbor Perspectives in Medicine*, *1*(1), a008862–a008862. https://doi.org/10.1101/cshperspect.a008862

Gonzalez-Rodriguez, P., Zampese, E., & Surmeier, D. J. (2020). Selective neuronal vulnerability in Parkinson's disease. In *Progress in brain research* (Vol. 252, pp. 61–89). Elsevier.

Gould, S. J. (2003). The Hedgehog, the Fox, and the Magister's Pox: Mending the Gap Between Science and the Humanities. Three Rivers Press. https://books.google.ca/books?id=LkMK-3L6hPQC

Greten-Harrison, B., Polydoro, M., Morimoto-Tomita, M., Diao, L., Williams, A. M., Nie, E. H., Makani, S., Tian, N., Castillo, P. E., Buchman, V. L., & Chandra, S. S. (2010). Aβγ-Synuclein triple knockout mice reveal age-dependent neuronal dysfunction. *Proceedings of the National Academy of Sciences*, *107*(45), 19573–19578. https://doi.org/10/dtv9ts

Gunn-Moore, D., Kaidanovich-Beilin, O., Gallego Iradi, M. C., Gunn-Moore, F., & Lovestone, S. (2018). Alzheimer's disease in humans and other animals: A consequence of postreproductive life span and longevity rather than aging. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, *14*(2), 195–204. https://doi.org/10/ggktnq

Heinzel, S., Berg, D., Gasser, T., Chen, H., Yao, C., & Postuma, R. B. (2019). Update of the MDS research criteria for prodromal Parkinson's disease. *Movement Disorders*, *34*(10), 1464–1470. https://doi.org/10/ggcb6s

Herculano-Houzel, S., Avelino-de-Souza, K., Neves, K., Porfírio, J., Messeder, D., Mattos Feijó, L., Maldonado, J., & Manger, P. R. (2014). The elephant brain in numbers. *Frontiers in Neuroanatomy*, *8*, 46. https://doi.org/10/gh25sf

Herculano-Houzel, S., Manger, P. R., & Kaas, J. H. (2014). Brain scaling in mammalian evolution as a consequence of concerted and mosaic changes in numbers of neurons and average neuronal cell size. *Frontiers in Neuroanatomy*, *8*, 77. https://doi.org/10/gh25r8

Hijaz, B. A., & Volpicelli-Daley, L. A. (2020). Initiation and propagation of α-synuclein aggregation in the nervous system. *Molecular Neurodegeneration*, *15*(1), 1–12. https://doi.org/10.1186/s13024-020-00368-6

Hornykiewicz, O. (2002). Dopamine miracle: From brain homogenate to dopamine replacement. *Movement Disorders: Official Journal of the Movement Disorder Society*, *17*(3), 501–508. https://doi.org/10/d743cc

Horsager, J., Knudsen, K., Andersen, K., Skj, C., Brooks, D., & Borghammer, P. (2019). "Brain first" vs. "Body first" Parkinson's disease is determined by RBD-status – a multi-modality imaging study. 2.

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., & Bohr, V. A. (2019). Ageing as a risk factor for neurodegenerative disease. *Nature Reviews Neurology*, *15*(10), 565–581. https://doi.org/10/gjvnnq

Huynh, B., Fu, Y., Kirik, D., Shine, J. M., & Halliday, G. M. (2021). Comparison of Locus Coeruleus Pathology with Nigral and Forebrain Pathology in Parkinson's Disease. *Movement Disorders*. https://doi.org/10.1002/mds.28615

Hyman, A. H., & Simons, K. (2011). Beyond HeLa cells. *Nature*, *480*(7375), 34–34. https://doi.org/10/b6s23k

Insel, T. R., Landis, S. C., & Collins, F. S. (2013). The NIH BRAIN Initiative. *Science (New York, N.y.)*, *340*(6133), 687–688. https://doi.org/10/gmkh2p

Iranzo, A., Fernández-Arcos, A., Tolosa, E., Serradell, M., Molinuevo, J. L., Valldeoriola, F., Gelpi, E., Vilaseca, I., Sánchez-Valle, R., Lladó, A., Gaig, C., & Santamaría, J. (2014). Neurodegenerative Disorder Risk in Idiopathic REM Sleep Behavior Disorder: Study in 174 Patients. *PLOS ONE*, *9*(2), e89741. https://doi.org/10/gdj53s

Jamieson, S. (2019). Shouldn't our expectations of students' and academics' intertextuality practices differ? In *Student plagiarism in higher education* (pp. 105–122). Routledge, Taylor & Francis Group.

Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neurology*, *53*(S3), S26–S38. https://doi.org/10.1002/ana.10483

Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *The Lancet*, *386*(9996), 896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Katz, P. (Ed.). (1999). *Beyond Neurotransmission: Neuromodulation and its Importance for Information Processing*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198524243.001.0001

Keane, M., Semeiks, J., Webb, A. E., Li, Y. I., Quesada, V., Craig, T., Madsen, L. B., van Dam, S., Brawand, D., Marques, P. I., Michalak, P., Kang, L., Bhak, J., Yim, H.-S., Grishin, N. V., Nielsen, N. H., Heide-Jørgensen, M. P., Oziolor, E. M., Matson, C. W., … de Magalhães, J. P. (2015). Insights into the Evolution of Longevity from the Bowhead Whale Genome. *Cell Reports*, *10*(1), 112–122. https://doi.org/10.1016/j.celrep.2014.12.008

Kikuchi, T., Maeda, K., Suzuki, M., Hirose, T., Futamura, T., & McQuade, R. D. (2021). Discovery research and development history of the dopamine D2 receptor partial agonists, aripiprazole and brexpiprazole. *Neuropsychopharmacology Reports*, *41*(2), 134–143. https://doi.org/10.1002/npr2.12180

Klein, A. D., & Mazzulli, J. R. (2018). Is Parkinson's disease a lysosomal disorder? *Brain: A Journal of Neurology*, *141*(8), 2255–2262. https://doi.org/10.1093/brain/awy147

Klein, C., & Westenberger, A. (2012). Genetics of Parkinson's Disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(1), a008888–a008888. https://doi.org/10.1101/cshperspect.a008888

Knopman, D. S., Amieva, H., Petersen, R. C., Chételat, G., Holtzman, D. M., Hyman, B. T., Nixon, R. A., & Jones, D. T. (2021). Alzheimer disease. *Nature Reviews Disease Primers*, *7*(1), 33. https://doi.org/10/gj2ns7

Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018, December 21). *Table 1, PARK-designated genes involved in familial Parkinson's disease* [Text]. Codon Publications. https://www.ncbi.nlm.nih.gov/books/NBK536722/table/Ch1-t0001/

Kulkarni, V. V., & Maday, S. (2018). Neuronal endosomes to lysosomes: A journey to the soma. *The Journal of Cell Biology*, *217*(9), 2977–2979. https://doi.org/10.1083/jcb.201806139

Lanciego, J. L., Luquin, N., & Obeso, J. A. (2012). Functional Neuroanatomy of the Basal Ganglia. *Cold Spring Harbor Perspectives in Medicine*, *2*(12). https://doi.org/10.1101/cshperspect.a009621

Langston, J. W. (2017). The MPTP Story. *Journal of Parkinson's Disease*, *7*(s1), S11–S19. https://doi.org/10.3233/JPD-179006

Lees, A. J., Selikhova, M., Andrade, L. A., & Duyckaerts, C. (2008). The black stuff and Konstantin Nikolaevich Tretiakoff. *Movement Disorders: Official Journal of the Movement Disorder Society*, *23*(6), 777–783. https://doi.org/10/dg8qtc

Li, S., & Le, W. (2017). Milestones of Parkinson's Disease Research: 200 Years of History and Beyond. *Neuroscience Bulletin*, *33*(5), 598–602. https://doi.org/10/gmzdt5

Liu, C., Goel, P., & Kaeser, P. S. (2021). Spatial and temporal scales of dopamine transmission. *Nature Reviews Neuroscience*, *22*(6), 345–358. https://doi.org/10.1038/s41583-021-00455-7

Liu, C., Kershberg, L., Wang, J., Schneeberger, S., & Kaeser, P. S. (2018). Dopamine Secretion Is Mediated by Sparse Active Zone-like Release Sites. *Cell*, *172*(4), 706-718.e15. https://doi.org/10.1016/j.cell.2018.01.008

Llinás, R. R. (2003). The contribution of Santiago Ramon y Cajal to functional neuroscience. *Nature Reviews Neuroscience*, *4*(1), 77–80. https://doi.org/10/fkj5ws

Lunt, W., Shah, M., Sang, J., Choi, J., Burke, S., Higgins, J., & Skene, N. (2021). Neurochemical identity and anatomy of cell loss in Parkinson's disease, and the clinical data available for correlation of progression: A systematic review and meta-analysis. https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021265515

Ma, S. Y., Longo, F. M., Röyttä, M., & Collan, Y. (2003). MODERN STEREOLOGICAL EVALUATION IN THE AGING HUMAN SUBSTANTIA NIGRA. *Image Analysis & Stereology*, *22*(2), 73–80. https://doi.org/10.5566/ias.v22.p73-80

Manaye, K. F., McIntire, D. D., Mann, D. M. A., & German, D. C. (1995). Locus coeruleus cell loss in the aging human brain: A non-random process. *Journal of Comparative Neurology*, *358*(1), 79–87. https://doi.org/10/fw65dx

Mandal, A., & Drerup, C. M. (2019). Axonal Transport and Mitochondrial Function in Neurons. *Frontiers in Cellular Neuroscience*, *13*, 373. https://doi.org/10.3389/fncel.2019.00373

Manger, P. (2019). Why study brains and behaviour of different mammals? *IBRO Reports*, *7*, 53. https://doi.org/10/gmvwvf

Manger, P. R. (2020). Consistencies and Variances in the Anatomical Organization of Aspects of the Mammalian Brain stem. In *Evolutionary Neuroscience* (pp. 377–396). Elsevier. https://doi.org/10.1016/B978-0-12-820584-6.00015-5

Marras, C., Beck, J. C., Bower, J. H., Roberts, E., Ritz, B., Ross, G. W., Abbott, R. D., Savica, R., Van Den Eeden, S. K., Willis, A. W., & Tanner, C. M. (2018). Prevalence of Parkinson's disease across North America. *Npj Parkinson's Disease*, *4*(1), 1–7. https://doi.org/10/gjwzff

Marras, C., & Lang, A. (2013). Parkinson's disease subtypes: Lost in translation? *Journal of Neurology, Neurosurgery & Psychiatry*, *84*(4), 409–415. https://doi.org/10/f4p6fh

Martínez-Cué, C., & Rueda, N. (2020). Cellular Senescence in Neurodegenerative Diseases. Frontiers in Cellular Neuroscience, 14. https://doi.org/10.3389/fncel.2020.00016

Marx, V. (2021). Method of the Year: Spatially resolved transcriptomics. *Nature Methods*, *18*(1), 9–14. https://doi.org/10.1038/s41592-020-01033-y

Matheoud, D., Cannon, T., Voisin, A., Penttinen, A.-M., Ramet, L., Fahmy, A. M., Ducrot, C., Laplante, A., Bourque, M.-J., Zhu, L., Cayrol, R., Campion, A. L., McBride, H. M., Gruenheid, S., Trudeau, L.-E., & Desjardins, M. (2019). Intestinal infection triggers Parkinson's disease-like symptoms in Pink1 −/− mice. *Nature*, *571*(7766), 565–569. https://doi.org/10.1038/s41586-019-1405-y

Matheoud, D., Sugiura, A., Bellemare-Pelletier, A., Laplante, A., Rondeau, C., Chemali, M., Fazel, A., Bergeron, J. J., Trudeau, L.-E., Burelle, Y., Gagnon, E., McBride, H. M., & Desjardins, M. (2016). Parkinson's Disease-Related Proteins PINK1 and Parkin Repress Mitochondrial Antigen Presentation. *Cell*, *166*(2), 314–327. https://doi.org/10.1016/j.cell.2016.05.039

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., & Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(2), 444–453. https://doi.org/10.1523/JNEUROSCI.4029-08.2009

Mechawar N, Cozzari C, Descarries L. 2000. Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. The Journal of Comparative Neurology 428:305–318. doi: 10.1002/1096-9861(20001211)428:23.0.CO;2-Y.

Mechawar N, Watkins KC, Descarries L. 2002. Ultrastructural features of the acetylcholine innervation in the developing rat parietal cortex. The Journal of Comparative Neurology 443:250–258. doi: 10.1002/cne.10114.

Mengler L, Khmelinskii A, Diedenhofen M, Po C, Staring M, Lelieveldt BP, Hoehn M. 2013. Brain maturation of the adolescent rat cortex and striatum: changes in volume and myelination. Neuroimage 84C:35–44. doi: 10.1016/j.neuroimage.2013.08.034.

McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology*, *38*(8), 1285–1285. https://doi.org/10/ggqqbp

Miettinen RA, Kalesnykas G, Koivisto EH. 2002. Estimation of the total number of cholinergic neurons containing estrogen receptor-alpha in the rat basal forebrain. The Journal of Histochemistry and Cytochemistry 50:891–902. doi: 10.1177/002215540205000703.

Microbe, T. L. (2021). Funding basic sciences: A solid structure is built from the ground up. *The Lancet Microbe*, *2*(2), e48. https://doi.org/10.1016/S2666-5247(21)00011-2

Moore, T. J., Glenmullen, J., & Mattison, D. R. (2014). Reports of pathological gambling, hypersexuality, and compulsive shopping associated with dopamine receptor agonist drugs. *JAMA Internal Medicine*, *174*(12), 1930–1933. https://doi.org/10.1001/jamainternmed.2014.5262

Muthane, U., Jain, S., & Gururaj, G. (2001). Hunting genes in Parkinson's disease from the roots. *Medical Hypotheses*, *57*(1), 51–55. https://doi.org/10.1054/mehy.2001.1289

Nadim, F., & Bucher, D. (2014). Neuromodulation of neurons and synapses. *Current Opinion in Neurobiology*, *29*, 48–56. https://doi.org/10/f6vb8p

Nalls, M. A., Blauwendraat, C., Vallerga, C. L., Heilbron, K., Bandres-Ciga, S., Chang, D., Tan, M., Kia, D. A., Noyce, A. J., Xue, A., Bras, J., Young, E., Coelln, R. von, Simón-Sánchez, J., Schulte, C., Sharma, M., Krohn, L., Pihlstrøm, L., Siitonen, A., … Zhang, F. (2019). Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: A meta-analysis of genome-wide association studies. *The Lancet Neurology*, *18*(12), 1091–1102. https://doi.org/10.1016/S1474-4422(19)30320-5

Nemri, A. (2010). Santiago Ramón y Cajal. *Scholarpedia*, *5*(12), 8577. https://doi.org/10/c6qwk6

Neurodegeneration. (2021). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=Neurodegeneration&oldid=1043688812

Nguyen, M., Wong, Y. C., Ysselstein, D., Severino, A., & Krainc, D. (2019). Synaptic, Mitochondrial, and Lysosomal Dysfunction in Parkinson's Disease. *Trends in Neurosciences*, *42*(2), 140–149. https://doi.org/10.1016/j.tins.2018.11.001

NICE. (2019). *Recommendations | Parkinson's disease in adults | Guidance | NICE*. https://www.nice.org.uk/guidance/ng71/chapter/Recommendations#pharmacological-management-of-motor-symptoms

NINDS. (2019). *Parkinson's Disease: Challenges, Progress, and Promise | National Institute of Neurological Disorders and Stroke*. https://www.ninds.nih.gov/Disorders/All-Disorders/Parkinsons-Disease-Challenges-Progress-and-Promise

O'Callaghan, C., Walpola, I. C., & Shine, J. M. (2021). Neuromodulation of the mind-wandering brain state: The interaction between neuromodulatory tone, sharp wave-ripples and spontaneous thought. *Philosophical Transactions of the Royal Society B*. https://doi.org/10.1098/rstb.2019.0699

Oliveria, S. F. (2018). The dark history of early deep brain stimulation. *The Lancet Neurology*, *17*(9), 748. https://doi.org/10.1016/S1474-4422(18)30237-0

Pacelli, C., Giguère, N., Bourque, M.-J., Lévesque, M., Slack, R. S., & Trudeau, L.-É. (2015). Elevated Mitochondrial Bioenergetics and Axonal Arborization Size Are Key Contributors to the Vulnerability of Dopamine Neurons. *Current Biology*, *25*(18), 2349–2360. https://doi.org/10.1016/j.cub.2015.07.050

Parent, A. (1997). The brain in evolution and involution. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire*, *75*(6), 651–667.

Parkinson, J. (1817). *An essay on the shaking palsy*. [London] : For Dawsons. http://archive.org/details/b28995776

Penders, B. (2018). Ten simple rules for responsible referencing. *PLoS Computational Biology*, *14*(4), e1006036. https://doi.org/10/gfrhwx

Peng, H., Xie, P., Liu, L., Kuang, X., Wang, Y., Qu, L., Gong, H., Jiang, S., Li, A., Ruan, Z., Ding, L., Yao, Z., Chen, C., Chen, M., Daigle, T. L., Dalley, R., Ding, Z., Duan, Y., Feiner, A., … Zeng, H. (2021). Morphological diversity of single neurons in molecularly defined cell types. *Nature*, *598*(7879), 174–181. https://doi.org/10.1038/s41586-021-03941-1

Perez SE, Dar S, Ikonomovic MD, DeKosky ST, Mufson EJ. 2007. Cholinergic forebrain degeneration in the APPswe/PS1DeltaE9 transgenic mouse. Neurobiology of Disease 28:3–15. doi: 10.1016/j.nbd.2007.06.015.

Pete Goss. (1998). *Close to the wind*. Headline Book Publishing.

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A.-E., & Lang, A. E. (2017a). Parkinson disease. *Nature Reviews Disease Primers*, *3*, 17013. https://doi.org/10.1038/nrdp.2017.13

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A.-E., & Lang, A. E. (2017b). Parkinson disease. *Nature Reviews Disease Primers*, *3*(1), 17013. https://doi.org/10.1038/nrdp.2017.13

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease. *Science*, *276*(5321), 2045–2047. https://doi.org/10/ft5x3g

Popejoy, A. B., & Fullerton, S. M. (2016). Genomics is failing on diversity. *Nature*, *538*(7624), 161–164. https://doi.org/10/gf4qsd

Postuma, R. B., & Berg, D. (2016). Advances in markers of prodromal Parkinson disease. *Nature Reviews Neurology*, *12*(11), 622–634. https://doi.org/10.1038/nrneurol.2016.152

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., Obeso, J., Marek, K., Litvan, I., Lang, A. E., Halliday, G., Goetz, C. G., Gasser, T., Dubois, B., Chan, P., Bloem, B. R., Adler, C. H., & Deuschl, G. (2015). MDS clinical diagnostic criteria for Parkinson's disease: MDS-PD Clinical Diagnostic Criteria. *Movement Disorders*, *30*(12), 1591–1601. https://doi.org/10.1002/mds.26424

Postuma, R. B., Bertrand, J.-A., Montplaisir, J., Desjardins, C., Vendette, M., Rios Romenets, S., Panisset, M., & Gagnon, J.-F. (2012). Rapid eye movement sleep behavior disorder and risk of dementia in Parkinson's disease: A prospective study. *Movement Disorders*, *27*(6), 720–726. https://doi.org/10.1002/mds.24939

Postuma, R. B., Poewe, W., Litvan, I., Lewis, S., Lang, A. E., Halliday, G., Goetz, C. G., Chan, P., Slow, E., Seppi, K., Schaffer, E., Rios-Romenets, S., Mi, T., Maetzler, C., Li, Y., Heim, B., Bledsoe, I. O., & Berg, D. (2018). Validation of the MDS clinical diagnostic criteria for Parkinson's disease. *Movement Disorders*, *33*(10), 1601–1608. https://doi.org/10/gfw54t

Prensa, L., & Parent, A. (2001). The nigrostriatal pathway in the rat: A single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *21*(18), 7247–7260.

Przedborski, S. (2017). The two-century journey of Parkinson disease research. *Nature Reviews Neuroscience*, *18*(4), 251–259. https://doi.org/10/gghd6r

Raghanti MA, Simic G, Watson S, Stimpson CD, Hof PR, Sherwood CC. 2011. Comparative analysis of the nucleus basalis of Meynert among primates. Neuroscience 184:1–15. doi: 10.1016/j.neuroscience.2011.04.008.

Raghanti MA, Stimpson CD, Marcinkiewicz JL, Erwin JM, Hof PR, Sherwood CC. 2008a. Cholinergic innervation of the frontal cortex: differences among humans, chimpanzees, and macaque monkeys. The Journal of Comparative Neurology 506:409–424. doi: 10.1002/cne.21546.

Raghanti MA, Stimpson CD, Marcinkiewicz JL, Erwin JM, Hof PR, Sherwood CC. 2008b. Differences in cortical serotonergic innervation among humans, chimpanzees, and macaque monkeys: a comparative study. Cerebral Cortex 18:584–597. doi: 10.1093/cercor/bhm089.

Reed, X., Bandrés-Ciga, S., Blauwendraat, C., & Cookson, M. R. (2019). The role of monogenic genes in idiopathic Parkinson's disease. *Neurobiology of Disease*, *124*, 230–239. https://doi.org/10.1016/j.nbd.2018.11.012

Rekdal, O. B. (2014). Academic Citation Practice: A Sinking Sheep? *Portal: Libraries and the Academy*, *14*(4), 567–585. https://doi.org/10.1353/pla.2014.0025

Rilling JK, Insel TR. 1999. The primate neocortex in comparative perspective using magnetic resonance imaging. Journal of Human Evolution 37:191–223. doi: 10.1006/jhev.1999.0313.

Rockenstein, E., Ostroff, G., Dikengil, F., Rus, F., Mante, M., Florio, J., Adame, A., Trinh, I., Kim, C., Overk, C., Masliah, E., & Rissman, R. A. (2018). Combined Active Humoral and Cellular Immunization Approaches for the Treatment of Synucleinopathies. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *38*(4), 1000–1014. https://doi.org/10.1523/JNEUROSCI.1170-17.2017

Rodriguez, M., Rodriguez-Sabate, C., Morales, I., Sanchez, A., & Sabate, M. (2015). Parkinson's disease as a result of aging. Aging Cell, 14(3), 293–308. https://doi.org/10.1111/acel.12312

Roser, M., & Ritchie, H. (2016). Burden of Disease. *Our World in Data*. https://ourworldindata.org/burden-of-disease

Rozenblatt-Rosen, O., Stubbington, M. J. T., Regev, A., & Teichmann, S. A. (2017). The Human Cell Atlas: From vision to reality. *Nature*, *550*(7677), 451–453. https://doi.org/10/gfgkr8

Sacchini, S., Díaz-Delgado, J., Espinosa de los Monteros, A., Paz, Y., Bernaldo de Quirós, Y., Sierra, E., Arbelo, M., Herráez, P., & Fernández, A. (2020). Amyloid-beta peptide and phosphorylated tau in the frontopolar cerebral cortex and in the cerebellum of toothed whales: Aging *vs* hypoxia. *Biology Open*, bio.054734. https://doi.org/10/gmvwwg

Santos, A. J., Asuncion, X. E., Rivero-Co, C., Ventura, M. E., Geronia II, R., Bangerter, L., & Sheils, N. E. (2021). Modeling differential rates of aging using routine laboratory data; Implications for morbidity and health care expenditure. *ArXiv:2103.09574 [Cs]*. http://arxiv.org/abs/2103.09574

Sarasa, M., & Gallego, C. (2006). Alzheimer-like neurodegeneration as a probable cause of cetacean stranding. *5th FENS Forum*. 5th FENS Forum, Vienna, Austria. https://scholar.google.com/citations?view_op=view_citation&hl=en&user=P27COtgAAAAJ&sortby=pubdate&citation_for_view=P27COtgAAAAJ:UeHWp8X0CEIC

Schapira, A. H. V. (2007). Mitochondrial dysfunction in Parkinson's disease. *Cell Death & Differentiation*, *14*(7), 1261–1266. https://doi.org/10/bp2rtg

Schekman, R., & Riley, E. A. (2019). Coordinating a new approach to basic research into Parkinson's disease. *ELife*, *8*, e51167. https://doi.org/10.7554/eLife.51167

Schwalb, J. M., & Hamani, C. (2008). The history and future of deep brain stimulation. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *5*(1), 3–13. https://doi.org/10.1016/j.nurt.2007.11.003

Seyhan, A. A. (2019). Lost in translation: The valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. *Translational Medicine Communications*, *4*(1), 18. https://doi.org/10.1186/s41231-019-0050-7

Shepherd, G. M. (2016). *Foundations of the neuron doctrine* (25th anniversary edition). Oxford University Press.

Shotton, D. (2010). CiTO, the Citation Typing Ontology. *Journal of Biomedical Semantics*, *1*(Suppl 1), S6. https://doi.org/10.1186/2041-1480-1-S1-S6

Si, Z., Sun, L., & Wang, X. (2021). Evidence and perspectives of cell senescence in neurodegenerative diseases. Biomedicine & Pharmacotherapy, 137, 111327. https://doi.org/10.1016/j.biopha.2021.111327

Simkin, M. V., & Roychowdhury, V. P. (2002). Read before you cite! *ArXiv:Cond-Mat/0212043*. http://arxiv.org/abs/cond-mat/0212043

Simón-Sánchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., Paisan-Ruiz, C., Lichtner, P., Scholz, S. W., Hernandez, D. G., Krüger, R., Federoff, M., Klein, C., Goate, A., Perlmutter, J., Bonin, M., Nalls, M. A., Illig, T., Gieger, C., … Gasser, T. (2009). Genome-wide association

study reveals genetic risk underlying Parkinson's disease. *Nature Genetics*, *41*(12), 1308–1312. https://doi.org/10/fp7xwn

Smith, R. (2002). In search of "non-disease." *BMJ : British Medical Journal*, *324*(7342), 883–885.

Spillantini, M. G., Schmidt, M. L., Lee, V. M.-Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-Synuclein in Lewy bodies. *Nature*, *388*(6645), 839–840. https://doi.org/10/c4vszd

Stern, G. (1989). Did parkinsonism occur before 1817? *Journal of Neurology, Neurosurgery & Psychiatry*, *52*(Suppl), 11–12. https://doi.org/10/df3nc7

Stokholm, M. G., Iranzo, A., Østergaard, K., Serradell, M., Otto, M., Svendsen, K. B., Garrido, A., Vilas, D., Borghammer, P., Santamaria, J., Møller, A., Gaig, C., Brooks, D. J., Tolosa, E., & Pavese, N. (2017). Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: A case-control study. *The Lancet Neurology*, *16*(10), 789–796. https://doi.org/10.1016/S1474-4422(17)30173-4

Südhof, T. C. (2012). The presynaptic active zone. *Neuron*, *75*(1), 11–25. https://doi.org/10.1016/j.neuron.2012.06.012

Südhof, T. C. (2018). Towards an Understanding of Synapse Formation. *Neuron*, *100*(2), 276–293. https://doi.org/10.1016/j.neuron.2018.09.040

Südhof, T. C. (2021). The cell biology of synapse formation. *Journal of Cell Biology*, *220*(7). https://doi.org/10.1083/jcb.202103052

Sulzer, D., Alcalay, R. N., Garretti, F., Cote, L., Kanter, E., Agin-Liebes, J., Liong, C., McMurtrey, C., Hildebrand, W. H., Mao, X., Dawson, V. L., Dawson, T. M., Oseroff, C., Pham, J., Sidney, J., Dillon, M. B., Carpenter, C., Weiskopf, D., Phillips, E., … Sette, A. (2017). T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature*, *546*(7660), 656–661. https://doi.org/10.1038/nature22815

Sulzer, D., & Edwards, R. H. (2019). The physiological role of α-synuclein and its relationship to Parkinson's Disease. *Journal of Neurochemistry*, *150*(5), 475–486. https://doi.org/10/gm4wgb

Surmeier, D. J., Obeso, J. A., & Halliday, G. M. (2017). Selective neuronal vulnerability in Parkinson disease. *Nature Reviews Neuroscience*, *18*(2), 101–113. https://doi.org/10.1038/nrn.2016.178

Surmeier, D. J., Schumacker, P. T., Guzman, J. D., Ilijic, E., Yang, B., & Zampese, E. (2017). Calcium and Parkinson's disease. *Biochemical and Biophysical Research Communications*, *483*(4), 1013–1019. https://doi.org/10.1016/j.bbrc.2016.08.168

Svalastog, A. L., & Martinelli, L. (2013). Representing life as opposed to being: The bio-objectification process of the HeLa cells and its relation to personalized medicine. *Croatian Medical Journal*, *54*(4), 397–402. https://doi.org/10/f2287x

Szaszi, K. (2015). A basic scientist's reflections on research funding. *Canadian Journal of Kidney Health and Disease*, *2*, 50. https://doi.org/10.1186/s40697-015-0087-0

Tanguay, W., Ducrot, C., Giguère, N., Bourque, M.-J., & Trudeau, L.-E. (2021). Neonatal 6-OHDA lesion of the SNc induces striatal compensatory sprouting from surviving SNc dopaminergic neurons without VTA contribution. *European Journal of Neuroscience*, *54*(7), 6618–6632. https://doi.org/10.1111/ejn.15437

Tansey, M. G., & Romero-Ramos, M. (2019). Immune system responses in Parkinson's disease: Early and dynamic. *European Journal of Neuroscience*, *49*(3), 364–383. https://doi.org/10.1111/ejn.14290

Travagli, R. A., Browning, K. N., & Camilleri, M. (2020). Parkinson disease and the gut: New insights into pathogenesis and clinical relevance. *Nature Reviews Gastroenterology & Hepatology*, *17*(11), 673–685. https://doi.org/10.1038/s41575-020-0339-z

Trétiakoff, C. (1919). Contribution a l'etude l'anatomie pathologique du locus Niger de soemmering: Avec quelques déductions relatives à la pathogénie des troubles du tonus musculaire et de la maladie de Parkinson. Jouve. https://books.google.ca/books?id=ySC0mgEACAAJ

Trudeau, L.-E. (2012). Obituary: Dr. Laurent Descarries (1939–2012). *Frontiers in Synaptic Neuroscience*, *4*, 7. https://doi.org/10.3389/fnsyn.2012.00007

Trudeau, L.-E., Hnasko, T. S., Wallén-Mackenzie, Å., Morales, M., Rayport, S., & Sulzer, D. (2014). The multilingual nature of dopamine neurons. *Progress in Brain Research*, *211*, 141–164. https://doi.org/10.1016/B978-0-444-63425-2.00006-4

Tyson, A. L., Rousseau, C. V., Niedworok, C. J., Keshavarzi, S., Tsitoura, C., Cossell, L., Strom, M., & Margrie, T. W. (2020). *A deep learning algorithm for 3D cell detection in whole mouse brain image datasets* [Preprint]. Neuroscience. https://doi.org/10.1101/2020.10.21.348771

Uéda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D. A., Kondo, J., Ihara, Y., & Saitoh, T. (1993). Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. *Proceedings of the National Academy of Sciences*, *90*(23), 11282–11286. https://doi.org/10/dt7s7s

Underwood MD, Khaibulina AA, Ellis SP, Moran A, Rice PM, Mann JJ, Arango V. 1999. Morphometry of the dorsal raphe nucleus serotonergic neurons in suicide victims. Biological Psychiatry 46:473–483. doi: 10.1016/ S0006-3223(99)00043-8.

Underwood MD, Mann JJ, Arango V. 2007. Morphometry of dorsal raphe nucleus serotonergic neurons in alcoholism. Alcoholism, Clinical and Experimental Research 31:837–845. doi: 10.1111/j.1530-0277.2007.00365.x.

United Nations, Department of Economic and Social Affairs, & Population Division. (2020). *World population ageing, 2019 highlights.*

Veronesi, U., Cascinelli, N., Mariani, L., Greco, M., Saccozzi, R., Luini, A., Aguilar, M., & Marubini, E. (2002). Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. *The New England Journal of Medicine*, *347*(16), 1227–1232. https://doi.org/10.1056/NEJMoa020989

Veronesi, U., Saccozzi, R., Del Vecchio, M., Banfi, A., Clemente, C., De Lena, M., Gallus, G., Greco, M., Luini, A., Marubini, E., Muscolino, G., Rilke, F., Salvadori, B., Zecchini, A., & Zucali, R. (1981). Comparing radical mastectomy with quadrantectomy, axillary dissection, and radiotherapy in patients with small cancers of the breast. *The New England Journal of Medicine*, *305*(1), 6–11. https://doi.org/10.1056/NEJM198107023050102

Vertes RP, Crane AM. 1997. Distribution, quantification, and morphological characteristics of serotonin immunoreactive cells of the supralemniscal nucleus (B9) and pontomesencephalic reticular formation in the rat. The Journal of Comparative Neurology 378:411–424. doi: 10.1002/(SICI)1096-9861(19970217)378:33.0.CO;2-6.

Vidyadhara, D. J., Lee, J. E., & Chandra, S. S. (2019). Role of the endolysosomal system in Parkinson's disease. *Journal of Neurochemistry*, *150*(5), 487–506. https://doi.org/10.1111/jnc.14820

von Chamier, L., Laine, R. F., Jukkala, J., Spahn, C., Krentzel, D., Nehme, E., Lerche, M., Hernández-Pérez, S., Mattila, P. K., Karinou, E., Holden, S., Solak, A. C., Krull, A., Buchholz, T.-O., Jones, M. L., Royer, L. A., Leterrier, C., Shechtman, Y., Jug, F., … Henriques, R. (2021).

Democratising deep learning for microscopy with ZeroCostDL4Mic. *Nature Communications*, *12*(1), 2276. https://doi.org/10.1038/s41467-021-22518-0

Wallings, R. L., Humble, S. W., Ward, M. E., & Wade-Martins, R. (2019). Lysosomal Dysfunction at the Centre of Parkinson's Disease and Frontotemporal Dementia/Amyotrophic Lateral Sclerosis. *Trends in Neurosciences*, *42*(12), 899–912. https://doi.org/10.1016/j.tins.2019.10.002

Wilson, S. (1925). The Croonian Lectures on some disorders of motility and of muscle tone: With special reference to the corpus striatum. *The Lancet*, *206*(5314), 1–10. https://doi.org/10/fdgj99

Winckler, B., Faundez, V., Maday, S., Cai, Q., Guimas Almeida, C., & Zhang, H. (2018). The Endolysosomal System and Proteostasis: From Development to Degeneration. *The Journal of Neuroscience*, *38*(44), 9364–9374. https://doi.org/10.1523/JNEUROSCI.1665-18.2018

Winnubst, J., Bas, E., Ferreira, T. A., Wu, Z., Economo, M. N., Edson, P., Arthur, B. J., Bruns, C., Rokicki, K., Schauder, D., Olbris, D. J., Murphy, S. D., Ackerman, D. G., Arshadi, C., Baldwin, P., Blake, R., Elsayed, A., Hasan, M., Ramirez, D., … Chandrashekar, J. (2019). Reconstruction of 1,000 Projection Neurons Reveals New Cell Types and Organization of Long-Range Connectivity in the Mouse Brain. *Cell*, *179*(1), 268-281.e13. https://doi.org/10.1016/j.cell.2019.07.042

Wong, Y. C., Luk, K., Purtell, K., Burke Nanni, S., Stoessl, A. J., Trudeau, L., Yue, Z., Krainc, D., Oertel, W., Obeso, J. A., & Volpicelli-Daley, L. A. (2019). Neuronal vulnerability in Parkinson disease: Should the focus be on axons and synaptic terminals? *Movement Disorders*, *34*(10), 1406–1422. https://doi.org/10.1002/mds.27823

Wu, H., Williams, J., & Nathans, J. (2014). Complete morphologies of basal forebrain cholinergic neurons in the mouse. *ELife*, *3*, e02444. https://doi.org/10.7554/eLife.02444

Yamamoto, K., & Vernier, P. (2011). The Evolution of Dopamine Systems in Chordates. *Frontiers in Neuroanatomy*, *5*, 21. https://doi.org/10/br9nx8

Zampese, E., & Surmeier, D. J. (2020). Calcium, Bioenergetics, and Parkinson's Disease. *Cells*, *9*(9), 2045. https://doi.org/10.3390/cells9092045

Zarow, C., Lyness, S. A., Mortimer, J. A., & Chui, H. C. (2003). Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases. *Archives of Neurology*, *60*(3), 337–341. https://doi.org/10.1001/archneur.60.3.337

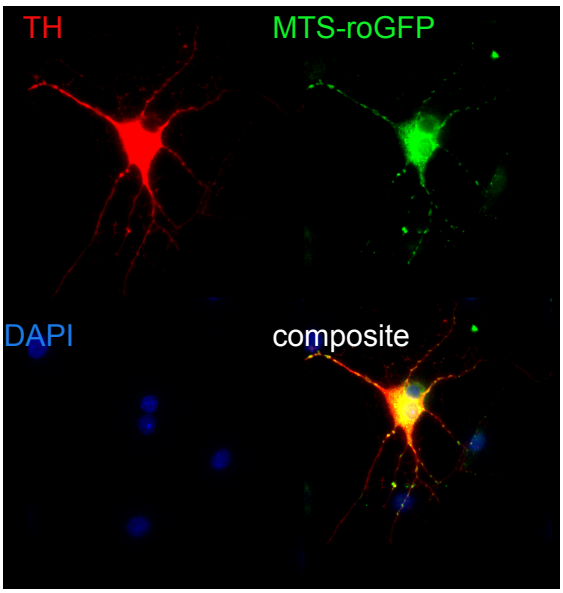
Zhang, Z.-X., Dong, Z.-H., & Román, G. C. (2006). Early descriptions of Parkinson disease in ancient China. *Archives of Neurology*, *63*(5), 782–784. https://doi.org/10/fwzzxr

Zweig, R. M., Cardillo, J. E., Cohen, M., Giere, S., & Hedreen, J. C. (1993). The locus ceruleus and dementia in Parkinson's disease. *Neurology*, *43*(5), 986–986. https://doi.org/10.1212/WNL.43.5.986

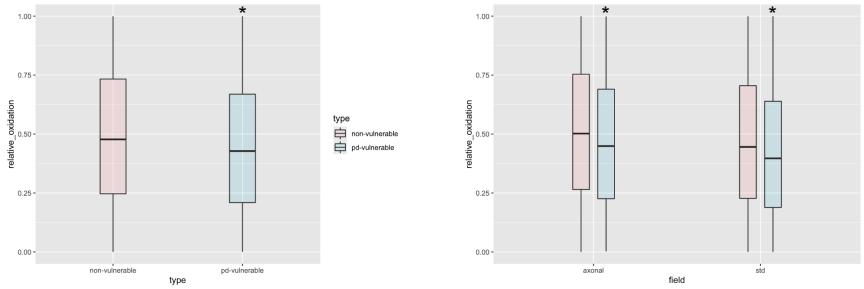
17 Appendix

Figure 1-2

A TH-GFP LC neurons infected with AAV9-TH-MTSroGFP



B No overt differences on relative oxidation between PD-vulnerable and resilient neurons



C