Parkinson's Disease

Far More than A Movement Disorder

Update on a Challenging Disease of Aging

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Introductory basics about Parkinson's disease

- 200+ years (!) since James Parkinson first described 'shaking palsy' as a movement disorder. PD still mostly diagnosed from Cardinal Triad of bradykinesia, resting tremor, and rigidity, affecting 2-3% ≥65 with PD.
- Although effective treatments available (L-dopa still a primary standard of care), disease burden over time is ↑ punitive, disease itself is still heavily depressogenic (via many potential mechanisms), and neuro-protection has been and remains an elusive treatment target (WEIN!)
- Outlook and prognosis for classical PD better than for most folks with Alzheimer's disease (not saying much!) but malignant forms of PD (diffuse Lewy body disease) may be significantly worse than AD.
- Non-motor symptoms have become increasingly a focus of concern & Rx due to significant impact on fxn/QOL:
  - Cognitive impairment (several phenotypes, ↓exec fxn/WM most common)
  - Autonomic and gastrointestinal dysfunction (PH, constipation)
  - Sleep disorders, especially REM sleep behavioral disorder
  - Mood disorders (principally depression but also hypomania)
  - Motivational difficulties (aside from attributable to depression)
  - Disorders of smell (anosmia)

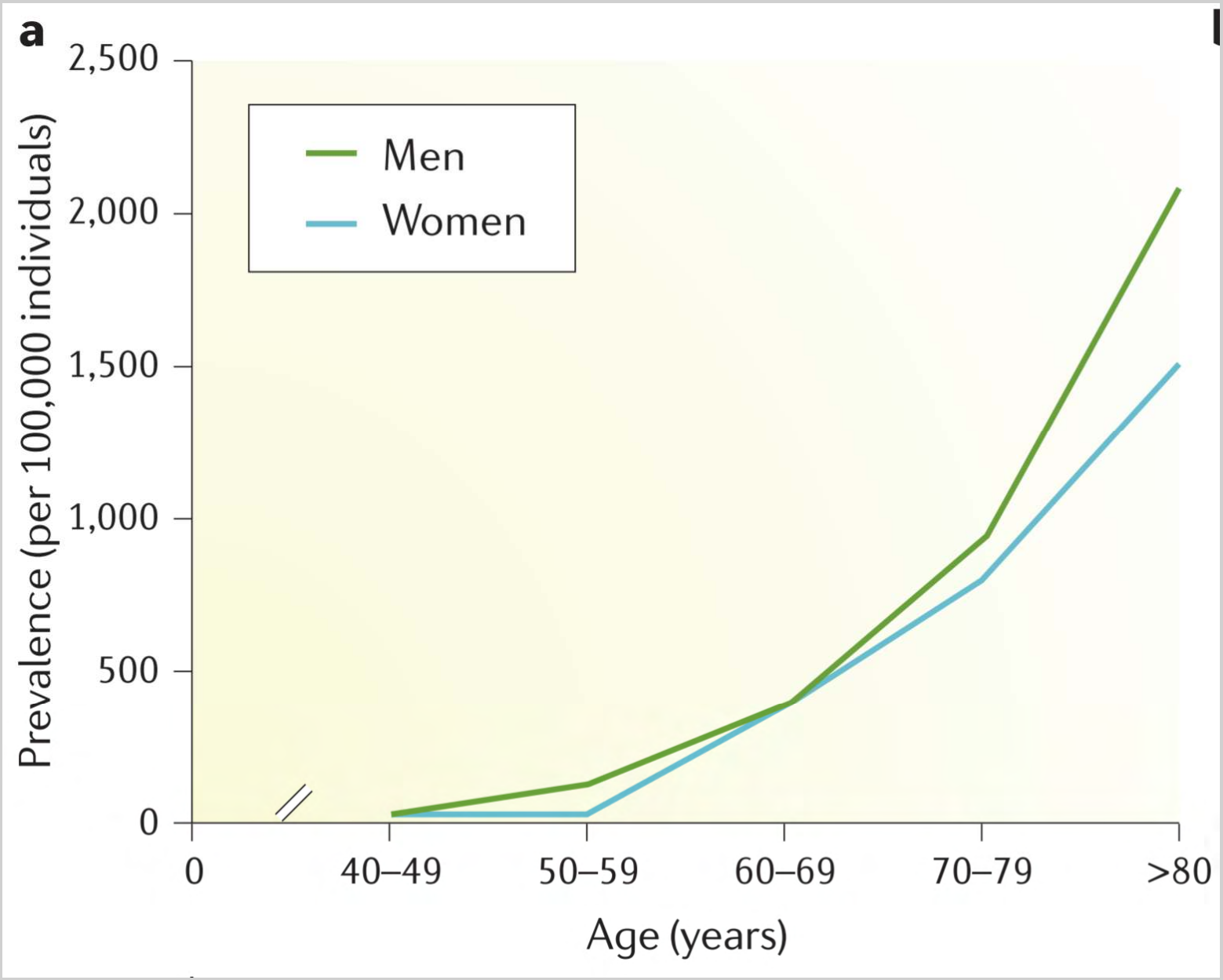
Basic Epidemiology

Incidence rates, implications for prevention.

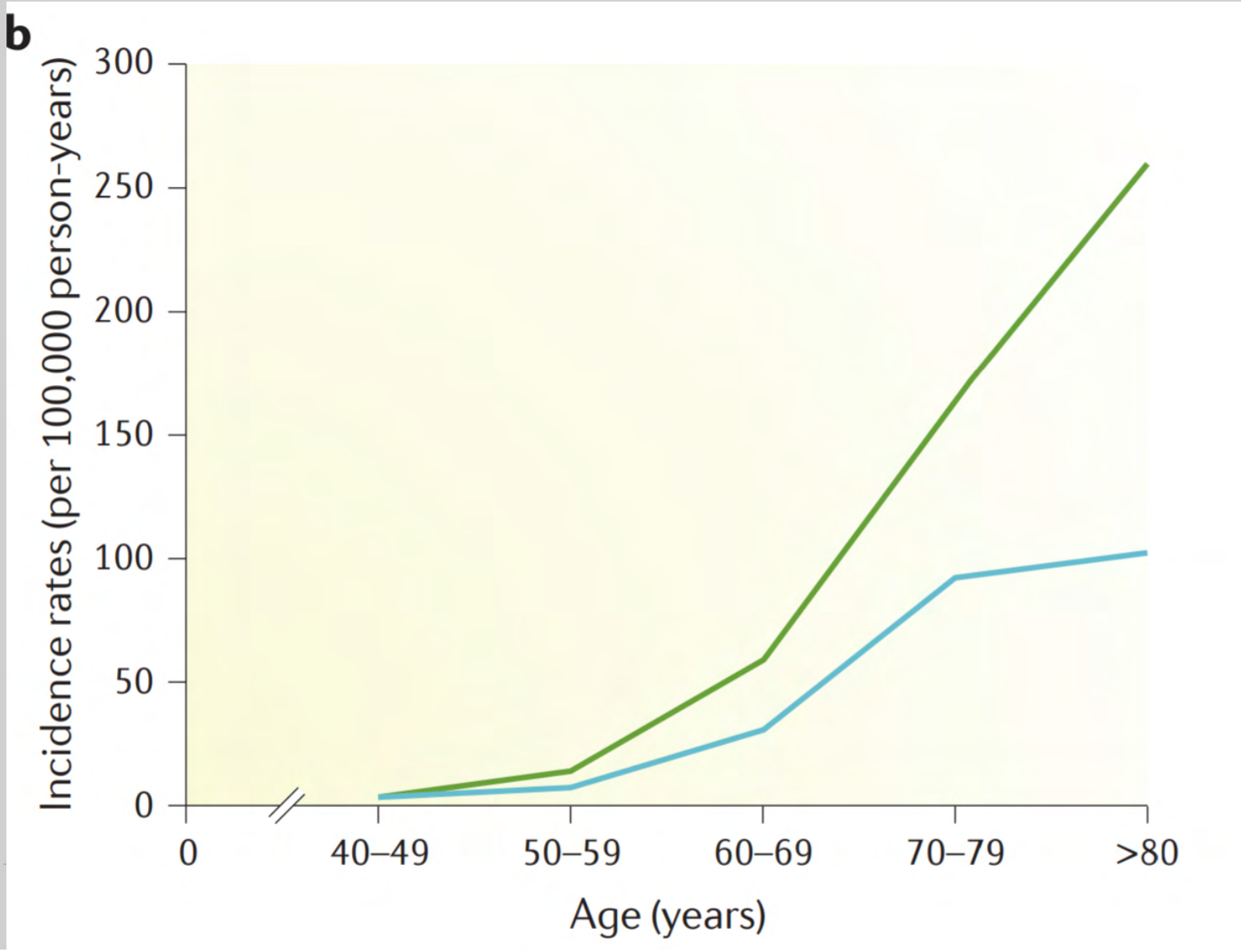
Basic Epidemiology

- Worldwide incidence estimates of PD are rather variable ~ between 5 to 35+ new cases per 100,000 individuals. Dietary/cultural modulation of risk? Curcuminoid-heavy diets may reduce risk x4.
- In many populations, PD almost twice as common in males vs females.
- PD is rare before age of 50 but ~5-10 fold increase from 50-80.
- Over 80, you have ~ 3% chance of some form of PD.
- From age 60+ PD→ ~ doubling of overall mortality rates, suggesting significant downstream (co?)morbidity. Exercise/activity decrements?
- Although not poised to break healthcare system like AD, aging demographics will significantly increase societal & healthcare burdens.
- Gene-environment interactions appear critical: history of TBI, ↑dairy products, pesticide/CNS toxin exposure, w/ lower incidence in smokers (nicotine) & coffee drinkers (many protective compounds).
- Prevention: healthy lifestyle practices, prevention of toxin exposure esp. pesticides/herbicides. Serum urate? Ibuprofen? CR mimetics?

Incidence of Parkinson's disease by age and sex



Graphing in person-years (Y Axis) exposes the risk separation between the genders. Basis?



Symptoms, diagnosis, histopathology and biomarkers:

Discriminating PD from DLBD and 'Parkinsonian Plus' Disorders

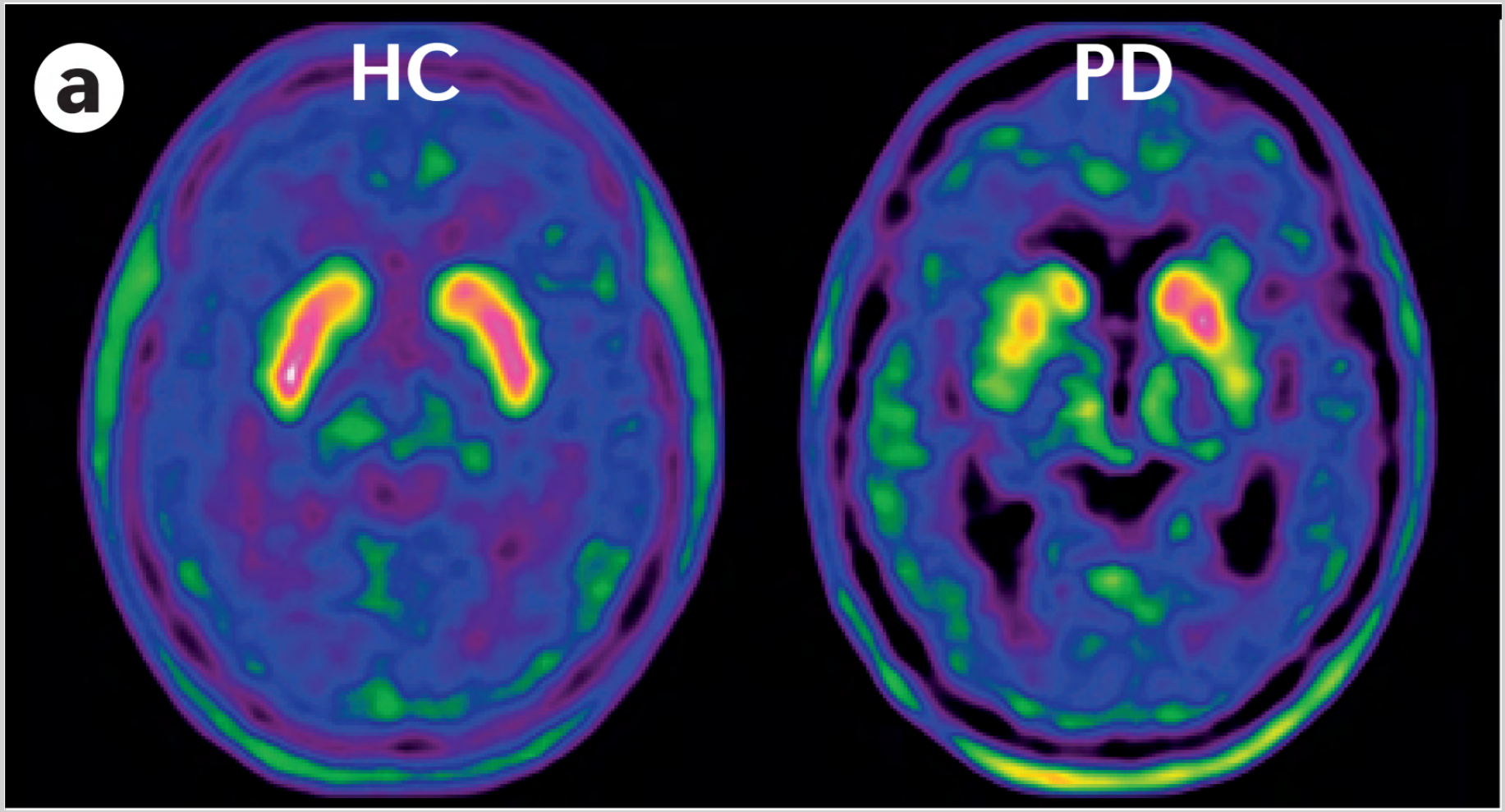
Confusing territory with complex correlations between histopathology, biomarkers and symptom phenotypes yielding much opportunity for diagnostic error.

International Parkinson and Movement Disorder Society Diagnostic Criteria for PD

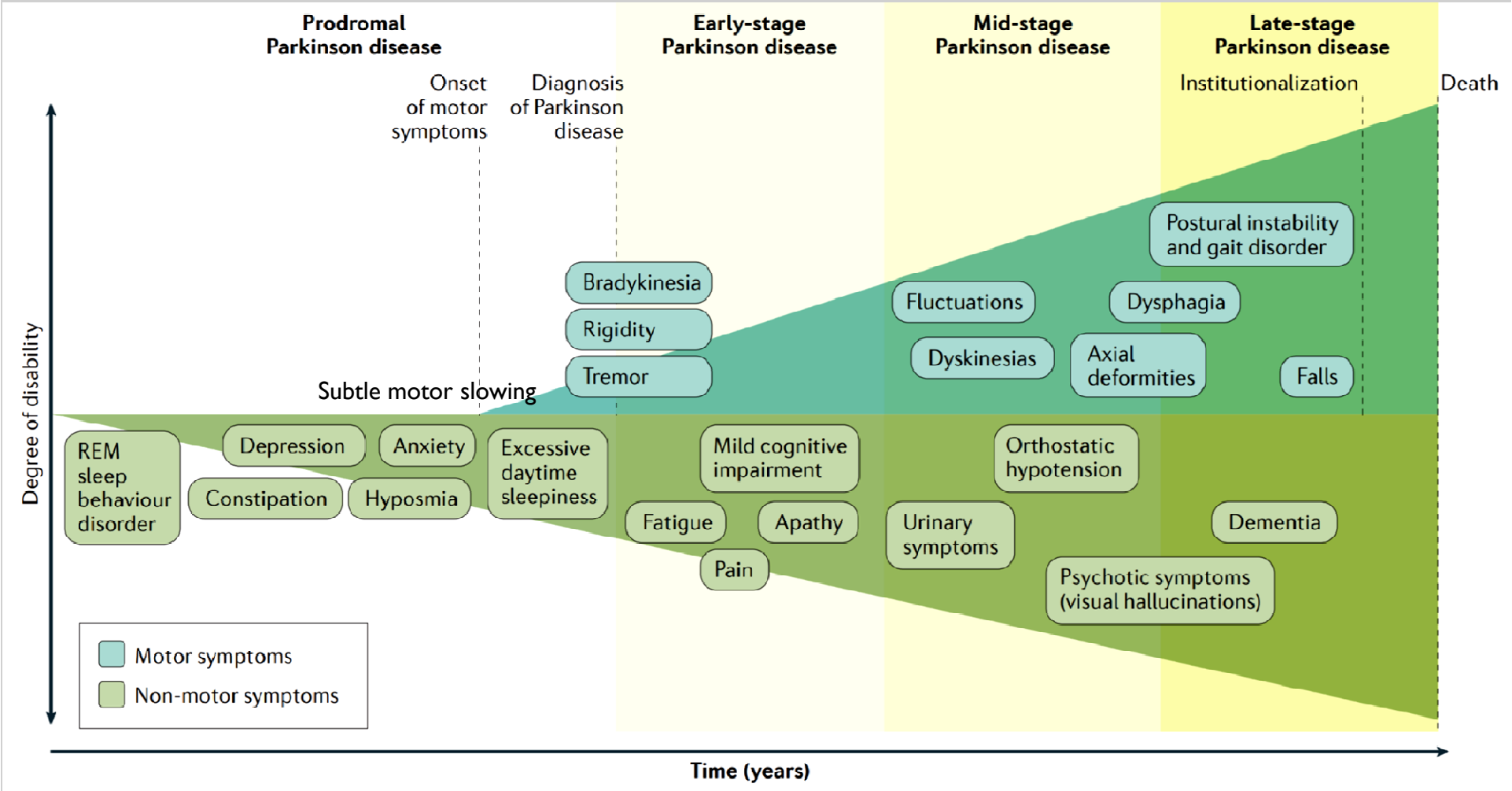
- **Step 1: *Diagnosis of parkinsonism*** (core feature)
  - Presence of bradykinesia, slowing of movement, w/decrement in amplitude or speed (or progressive hesitations or halts) as movements are continued.
  - In combination with at least one of: rigidity and/or resting tremor.
- **Step 2:** determining PD as probable cause of parkinsonism with two levels of diagnostic certainty. Diagnosis of ***clinically established Parkinson's disease*** *requires meeting all 3 following parameters*:
  1) **Absence of absolute exclusion criteria**: No clinical or imaging evidence for alternate etiology for parkinsonism (*drug-induced parkinsonism or essential tremor)*
  2) **Two or more supportive criteria**: including *L-DOPA responsiveness, presence of classic resting tremor, presence of l-DOPA-induced dyskinesias, presence of olfactory loss or cardiac sympathetic denervation on metaiodobenzylguanidine scintigraphy*.
  3) **No 'red flags'.** Features that are unusual but not absolutely exclusionary for Parkinson's disease, i.e., rapid progression of gait impairment or severe falls → wheelchair use (PSP?), or severe autonomic failure 3-5 years after onset (MSA?), rapid cognitive collapse (DLBD), etc.

- Diagnosis of ***clinically probable Parkinson's disease***: Absence of ***absolute exclusion criteria*** (noted above) but with presence of at least one ***red flag*** (as above) that are counterbalanced by ***supportive criteria***.

^{18}Fluorine L-dopa Imaging of basal ganglia – early to mid clinical stage – note asymmetry of DA loss (Poewe et al, 2018 NRDP)



Temporal organization of Parkinson's sxns and disease progression (classical PD, not DLBD)



Diagnosis of PD occurs w/onset of classic motor sxns (early-stage PD), typically in 50s, preceded by prodromal phase of years or even decades, characterized by non-motor symptoms (prodromal PD). Non-motor symptoms ↑prevalent & obvious over course of illness, but variably present throughout all stages. Progressive disability driven by these non-motor problems & ↑severity of cardinal motor features, ↑L-DOPA-related motor complications (on-off & dyskinesias) & evolution of L-DOPA-refractory motor disabilities (postural instability, gait problems (esp. freezing/falls) & dysphagia (late-stage).

Diffuse Lewy body disease (***DLBD***) – a malignant form of PD w/'phenotypic inversion'

- Diffuse Lewy body disease, unfortunately often referred to as "***Dementia w/Lewy bodies***" (DLB), is rapidly progressing neurocognitive disorder, w/Lewy bodies more widely distributed, in many autonomic, limbic/subcortical, and neocortical locations.
- Distinguishing DLB from PD: early-onset and rapid progression of cognitive issues, visual hallucinations, responses to DA Rx (poorer) & anti-psychotics, & imaging.
- ***"Rock and a hard place" pharmacology*** – DA agonists/L-dopa: ↑ risk of inducing psychosis, while neuroleptics severely exacerbate Parkinsonianism. MDs chasing own tails, w/↑opportunities for iatrogenesis (was common to find in chart reviews).
- Clinical features: rapidly progressive dementia → confusional presentation after only a few years, often with significant daily fluctuations, visual hallucinations/other psychotic symptoms, variable parkinsonism (sometimes appearing *after* cognitive symptoms), w/common REM sleep behavior disorder. ***Rapidly disabling.***
- Extensive involvement of ACh basal forebrain, w/more severe cholinergic deprivation of forebrain than in AD, consistent with typically positive response to cholinesterase inhibition (regarded as the default Rx option – ***start ASAP***).
- Very high % of AD histopathology in DLBD, ? Is it midway between AD & PD?
- Structural imaging: reduced amygdala volume. SPECT/PET: abnormal glucose perfusion/metabolism in *parietal & occipital regions*. No biofluid-based biomarkers yet.

What are ***Parkinson's Plus Syndromes?***

- Parkinson's plus syndromes: neurodegenerative disorders with significant symptom overlap with PD (parkinsonianism!) and are often misdiagnosed as PD as a result. Diagnosis (sometimes) corrected as these separate from a more classic PD trajectory (bilateral vs. unilateral). Typically have both poor response to L-Dopa, & sxns that are so-called 'red flags' in prior criteria set.
  - **Progressive supranuclear palsy (PSP).** PSP causes severe trouble with balance & stability (doesn't appear until late in PD w/postural instability). Doesn't show resting tremor, more difficulty w/eye movement (esp. vertical gaze control) and more trouble w/ speech, swallowing, and mood than people with classical PD.
  - **Multiple system atrophy (MSA).** MSA affects autonomic fxn > PD. It does show stiffness and loss of balance but also typically w/out resting tremor. A progressive dysautonomia impacts digestion, breathing, and cardiac regulation, w/ typically marked orthostatic hypotension (also seen in PD) (when severe it can index Shy-Drager syndrome, which can precede MSA/BG symptoms).
  - **Corticobasal (ganglionic) degeneration (CBGD).** CBD can show tremors, balance and cognitive problems, esp. apraxia. Over time, CBD →difficulty with speaking & writing. Can show paretic difficulties, and even ALS-like phenotypes.
  - **Lewy body dementia (LBD).**

Table 1. Parkinsonian disorders

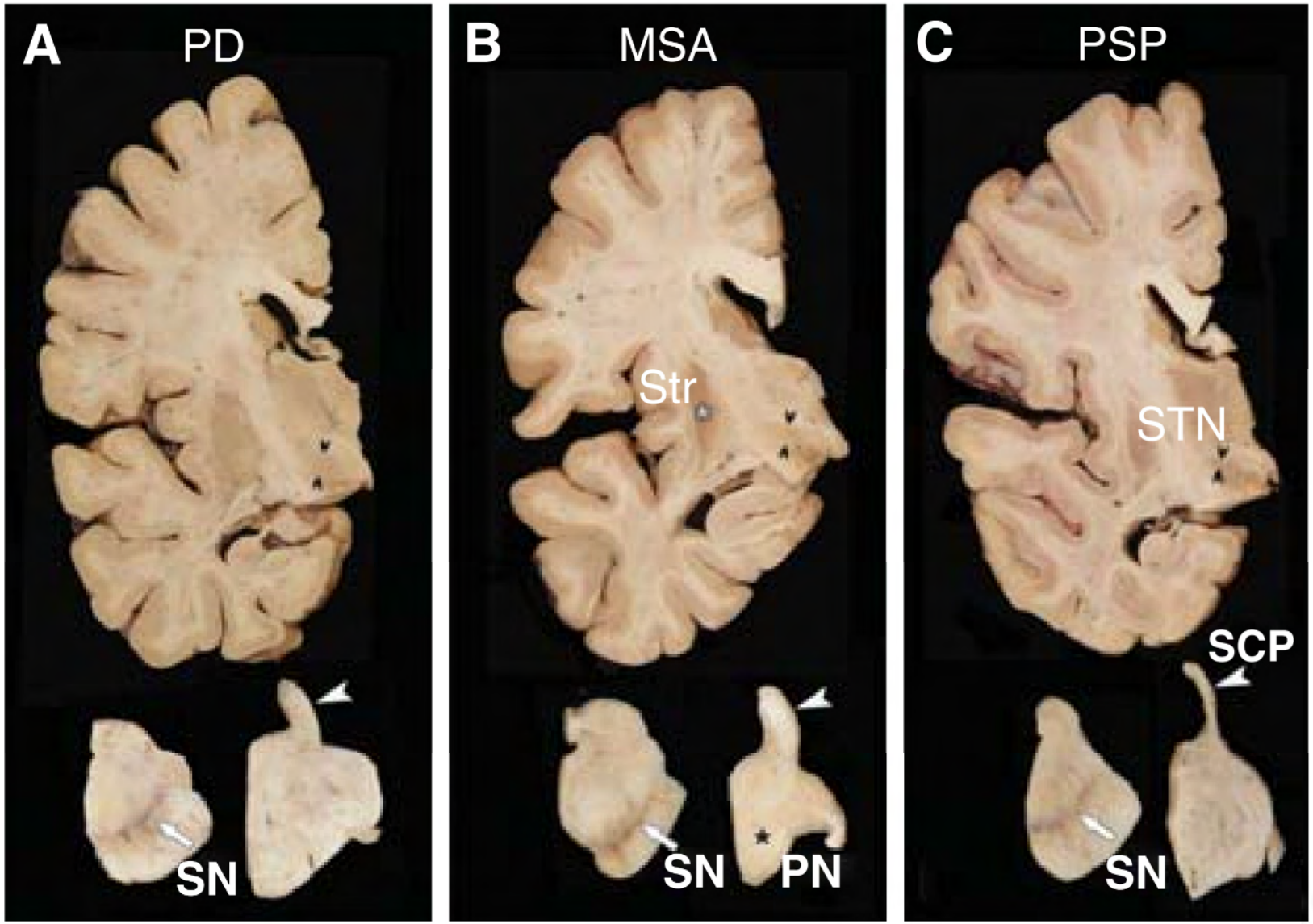
Classification	Examples
Degenerative parkinsonism	
α-Synuclein	Parkinson's disease, multiple system atrophy
Tau	Progressive supranuclear palsy, corticobasal degeneration, Guam parkinson dementia complex, chronic traumatic encephalopathy
TDP-43	Frontotemporal lobar degeneration (FTLD-TDP)
Nondegenerative parkinsonism	
Vascular	Vascular parkinsonism
Toxic	MPTP, manganese poisoning
Drug-induced	Antipsychotic medications
Infectious	Influenza virus (postencephalitic parkinsonism)

Parkinsonianism can be neurodegenerative or non-degenerative, and neurodegenerative forms can show three basic histopathologies/ proteinopathies. PSP & MSA have bilateral, PD unilateral onset of sxns.

Most common by far is drug-induced Parkinsonianism from neuroleptics, but other toxicities can also severely & permanently degrade midbrain DA systems. (Dickson, 2012)

Basic Neuropathology

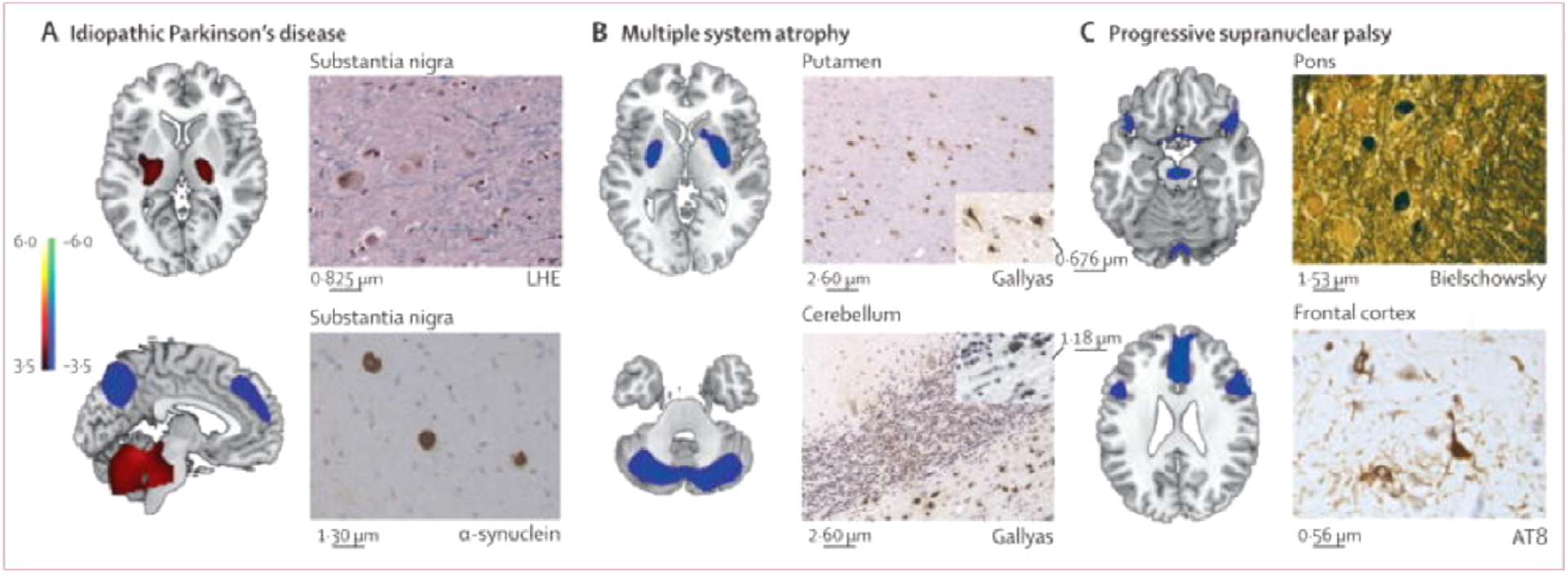
- Neuronal loss in substantia nigra, esp. in lateral-ventral tier of SN, w/curious relative preservation of ventral tegmental area (VTA – adjacent dopamine fields w/different projection targets (MLMC).
- Loss of SN DA→neostriatum begins preclinically, already quite advanced by time of onset of visible motor sxns (30-35% loss).
- Intracellular accumulations of α-synuclein (Presynaptic plasticity protein?). α-synuclein aggregates→ Lewy bodies (LB).
- In classical PD, LB generally confined to brainstem early (cholinergic, aminergic & olfactory areas), but in DLBD, LB are widespread early.
- Neither of these (protein aggregates of alpha synuclein and SN DA loss) individually is definitive or unique to PD. LB seen in other NDD.
- Also, LB don't predict degree of neuronal loss, and spread of LB doesn't seem to conform neatly to a simple network connectivity model, but may have to do with UK neural phenotypic vulnerabilities.
- Interesting question about two phenotypes, diffuse versus classical Lewy bodies. Clear bifurcation or a continuum? Do extra LB kindle AD-type process given high % of AD pathology in DLBD?



(Dickson 2012 – Anatomic differences in regional atrophy across various subcortical structures – pons in MSA, STN and SCP in PSP. All 3 show loss of pigment in SN DA cell groups

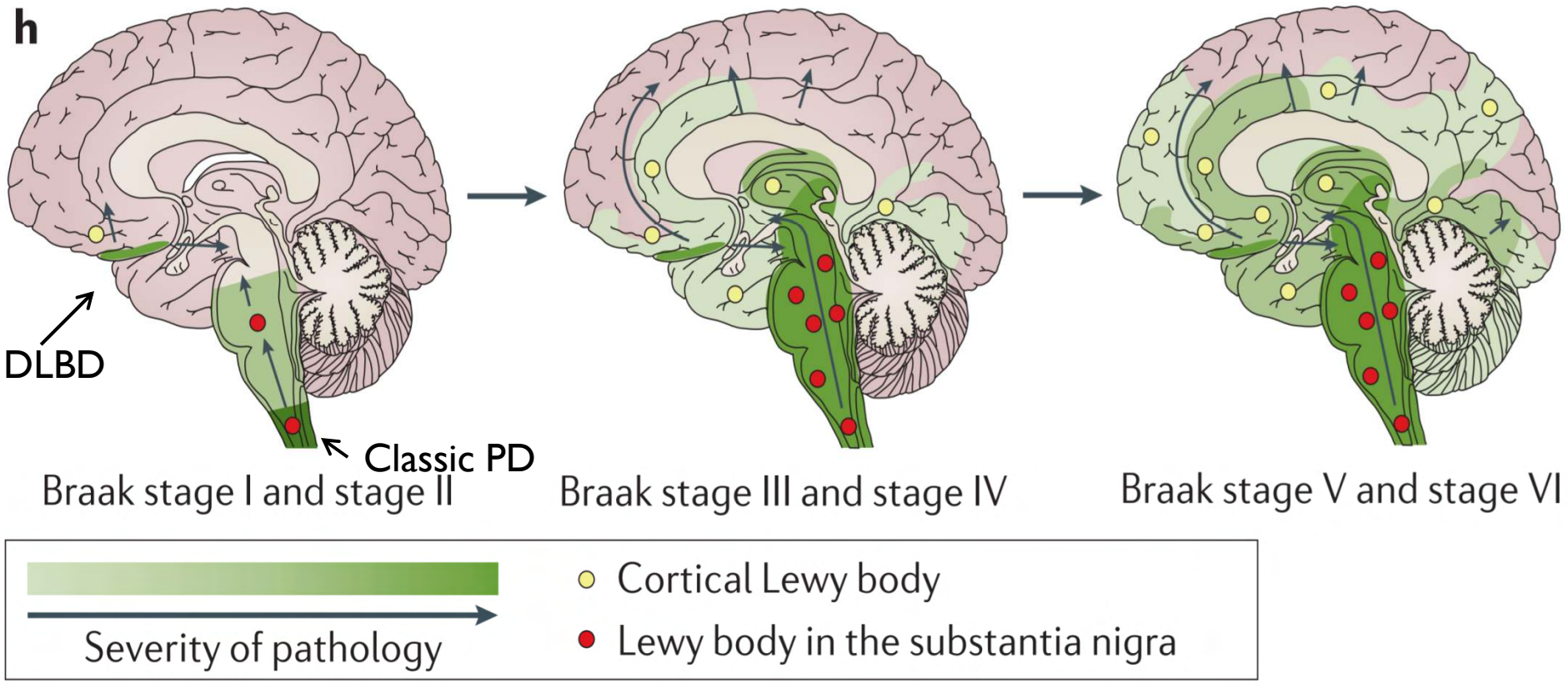
Transverse sections of midbrain (lower left) & pons (lower right) show pigment loss in SN (white arrows) in all 3 disorders, correlating w/parkinsonism. MSA shows atrophy of pontine base (black asterisk in **B**), whereas pontine base is unremarkable in PD & PSP. In PSP, superior cerebellar peduncle (marked w/white arrowheads **A**–**C**) has marked atrophy whereas it has normal thickness in PD and MSA. STN (subthalamic nucleus) is normal in PD (**A**) & MSA (**B**) but shows atrophy in PSP (**C**) (double black arrowheads = widest diameter of STN). MSA shows atrophy & dark discoloration of posterior putamen (white asterisk in B), but no atrophy or discoloration is noted in PD or PSP.

Metabolic PET imaging can discriminate PD from PSP and multiple system atrophy.



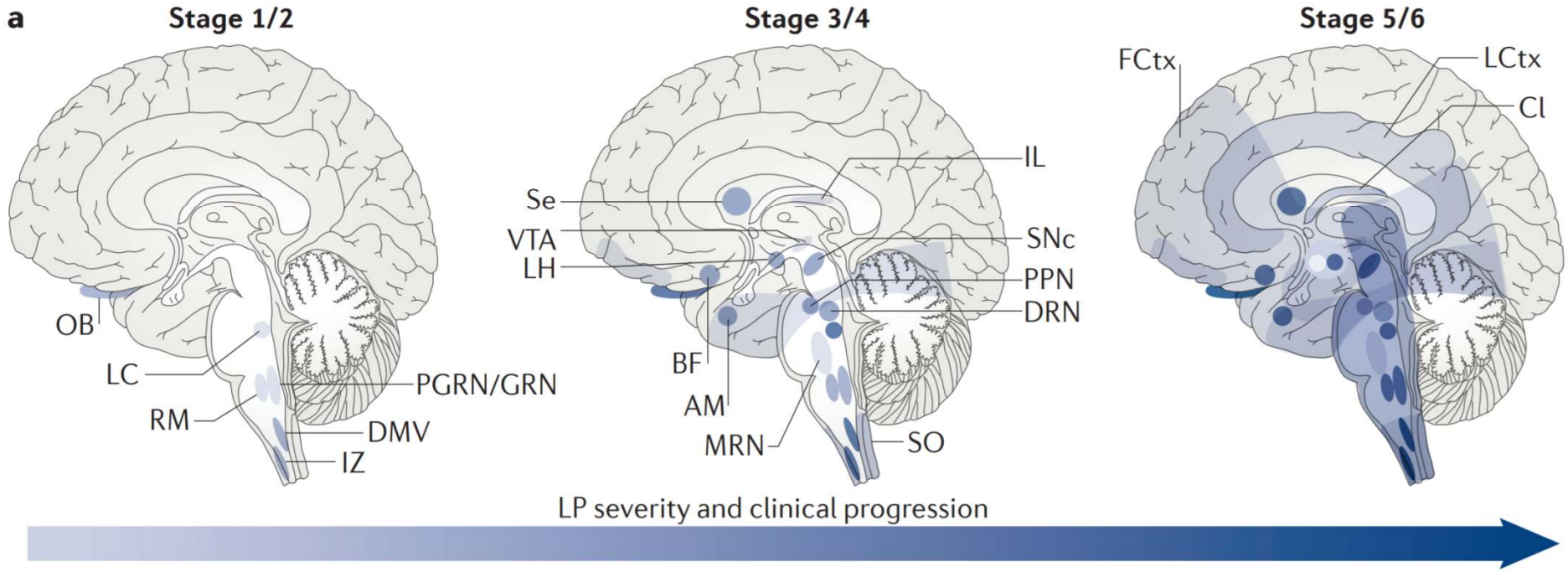
(**A)** The PET pattern in idiopathic PD shows increased (red) pallidothalamic and pontocerebellar metabolic activity associated w/relative reductions (blue) in premotor cortex, supplementary motor area, & parietal association regions. **(B)** Multiple system atrophy-related PET pattern shows bilateral metabolic reductions in putamen & cerebellar activity, paired w/neuronal loss & gliosis in putamen (top) & cerebellum (bottom). Both regions displayed glial cytoplasmic inclusions. **(C)** The progressive supranuclear palsy pattern shows metabolic reductions in upper brainstem, medial frontal cortex, and medial thalamus, paired w/tangles in basis pontis, and tangles w/cytoplasmic inclusions & neuropil threads in fifth cortical layer of the prefrontal region. (Images from Tang et al., 2010)

Classic Braak & Braak staging of PD & LBs



h | Theorized progression of α-syn aggregation in PD w/out concomitant AD pathology. α-Synuclein inclusions in olfactory, cholinergic & monoaminergic lower brainstem neurons in asymptomatic cases (Braak stage I/II), infiltrate ACh, DA?NE neurons in midbrain & basal forebrain in those with classic motor sxns of PD (Braak stage III/IV), and then are found later in limbic and neocortical brain regions w/ disease progression (Braak stage V/VI) (from Poewe, et al, 2017 w/ DW edits). However, Braak stages correlate poorly w/ degree of SN DA loss, suggesting ***mechanistic separation btw spread of proteinopathy & neurodegeneration***.

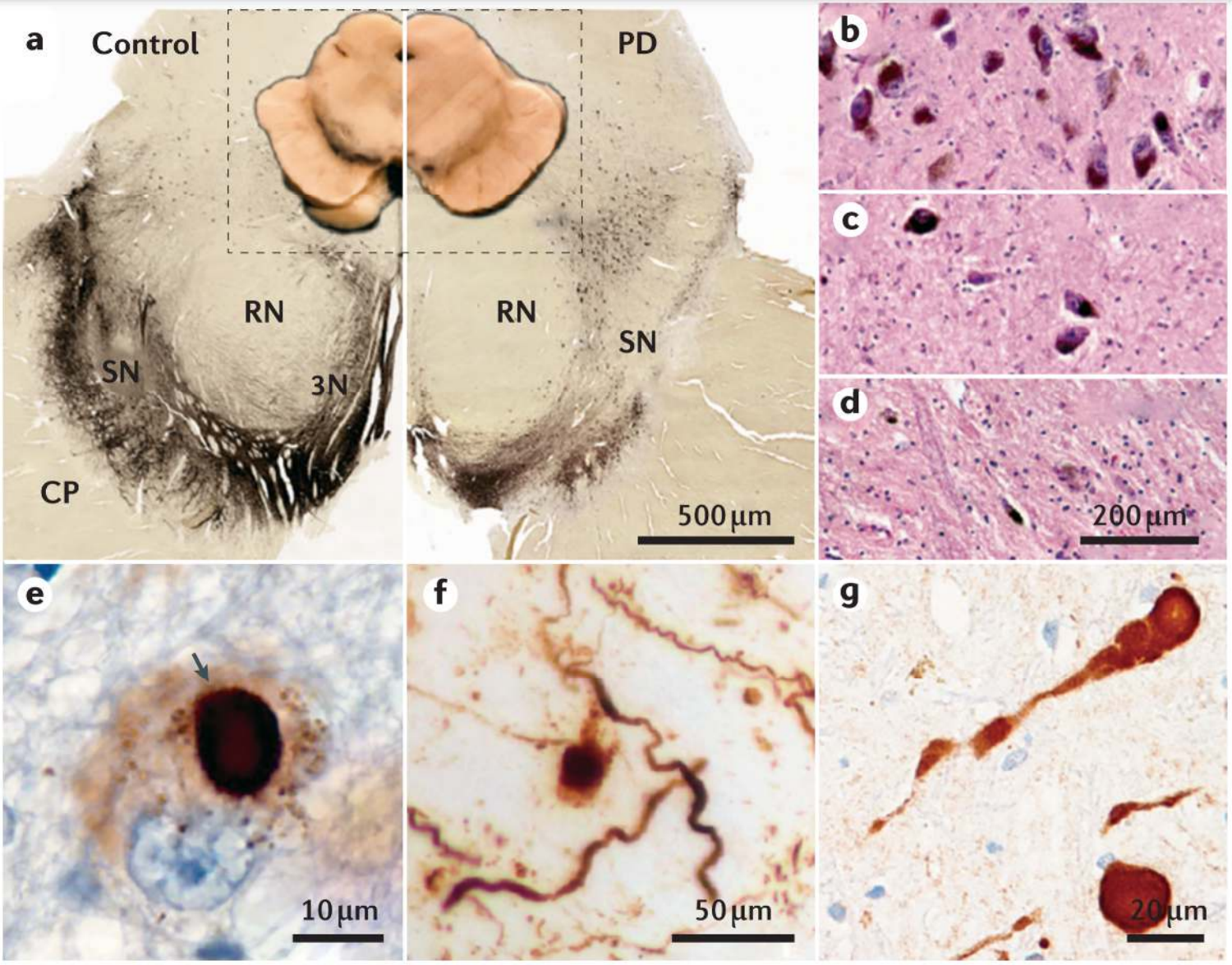
More fine grained staging and progression of LB



What defines these many regions aside from RAS connectivity making them vulnerable to LB pathology is still unclear. Connectivity alone doesn't appear to explain emerging patterns. ~50% w/ PD show distribution of LP consistent w/classic Braak staging model. While still impressive, this finding argues that, whereas CNS areas susceptible to LP are well defined, the sequence in which LBs are manifested, and their extent, is not well understood. (Surmeier et al, 2017 NRN).

AM, amygdala; BF, magnocellular nuclei of the basal forebrain; CI, claustrum; DMV, dorsal motor nucleus of vagus; DRN, dorsal raphe nucleus; FCtx, frontal cortex; IZ, intermediate reticular zone; LC, locus coeruleus; LCtx, limbic cortex; LH, lateral hypothalamus; MRN, median raphe nucleus; OB, olfactory bulb; PGRN/GRN, paragigantocellular/gigantocellular reticular nucleus; PPN, pedunculopontine nucleus; RM, raphe magnus; Se, septum; SNc, substantia nigra pars compacta; SO, solitary tract nuclei; VTA, ventral tegmental area.

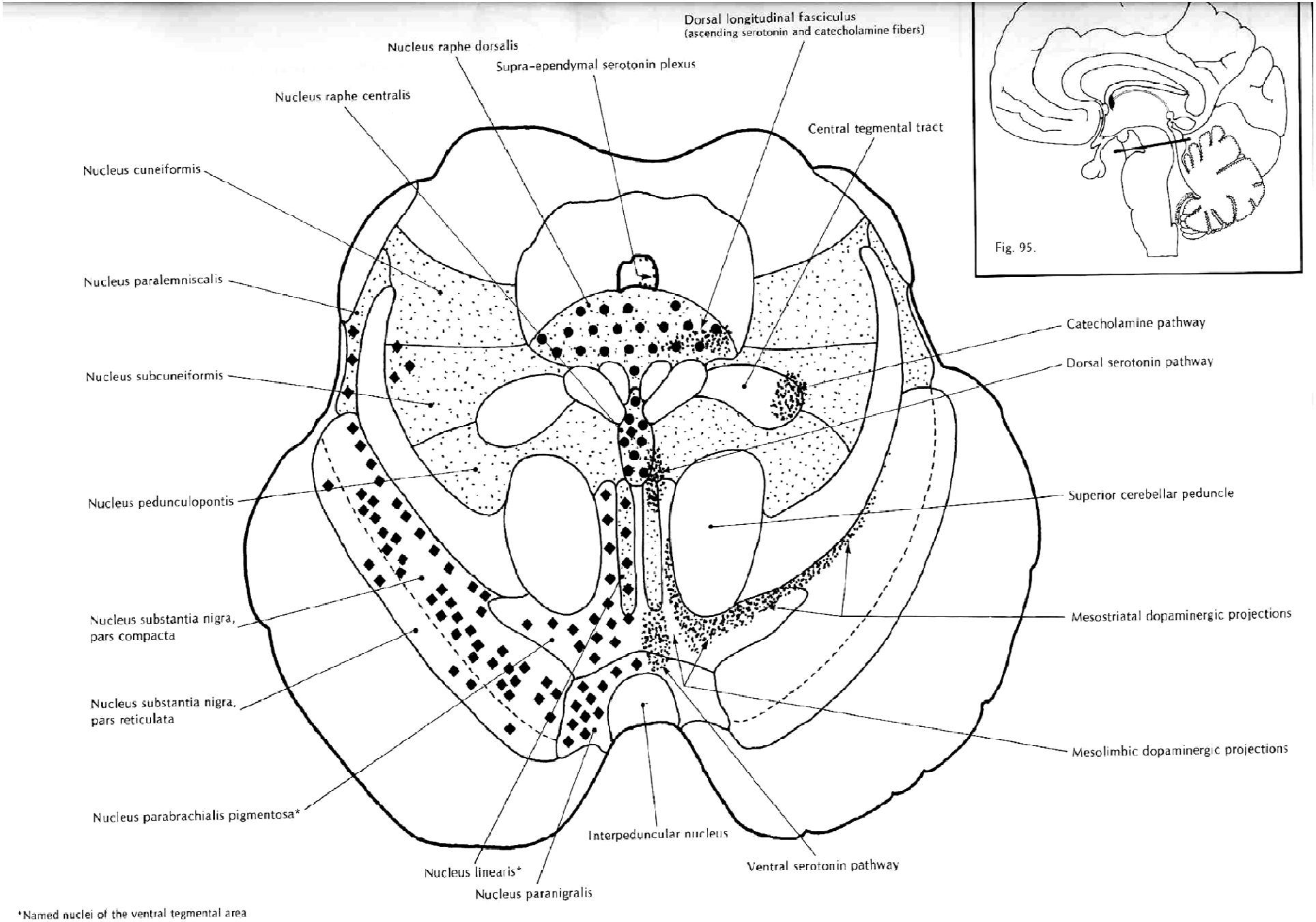
Histopathology w/SN DA loss and Lewy bodies
(Poewe et al, 2017 NRDP)



a stain for tyrosine hydroxylase (RLE for DA) –heavy loss in lateral/ ventral tier of SN

b-d Haematoxylin & eosin staining of VL SN showing distribution of pigmented neurons in a healthy control (**b**), diagnostically significant moderate (**c**) or severe (**d**) DA cell loss.

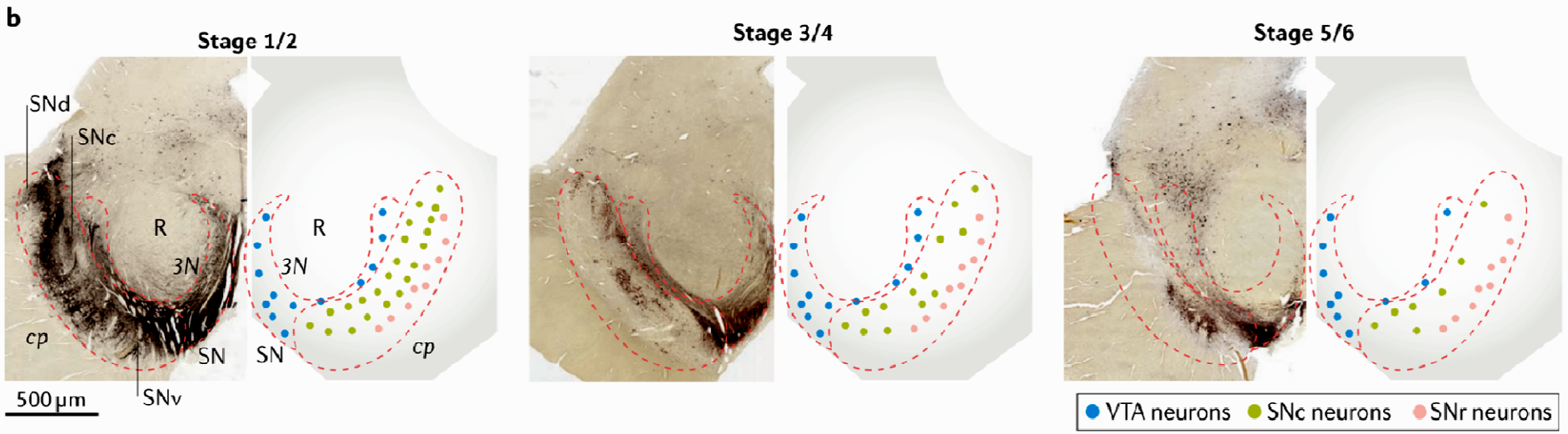
e-g Immunohistochemical staining of α-synuclein shows round, intra-cytoplasmic Lewy bodies (arrow in **e**), more diffuse, granular deposits of α-synuclein (**e** & **f**), deposits in neuronal cell processes (**f**), extra-cellular dot-like α-synuclein structures (**f**), α-synuclein spheroids in axons (**g**)



*Named nuclei of the ventral tegmental area

Figure 95. Midbrain: Level between inferior and superior colliculi. Diamonds, dopaminergic neurons; circles, serotonergic

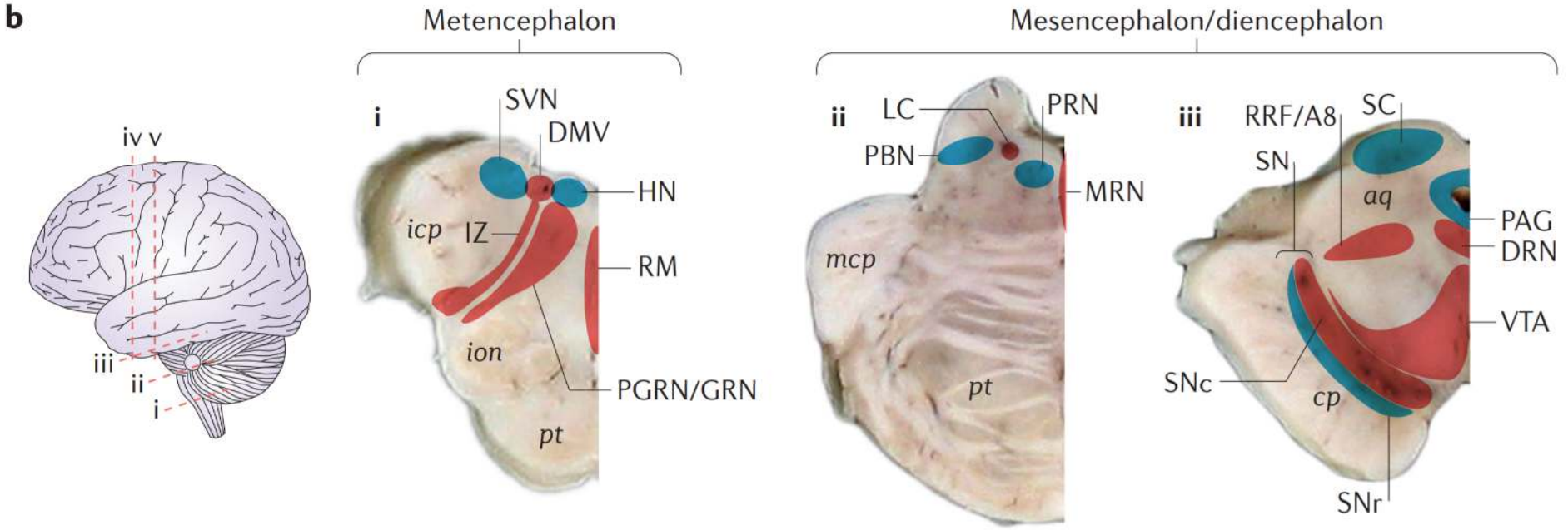
Histopathological progression of SN DA loss



Transverse sections of midbrain; the normal distribution of tyrosine hydroylase-immunopositive dopaminergic (DA) neurons shown in left panels, and pattern is schematized in the right panels. Heavily pigmented neurons of substantia nigra pars compacta (SNc) depicted in green; less pigmented neurons of ventral tegmental area (VTA) depicted in blue; neurons of SN pars reticulata (SNr) depicted in pink. Initial loss of ventral-tier SNc observed in patients with stage 4 depicted in middle panel, w/ greater cell loss observed over time at later stages.

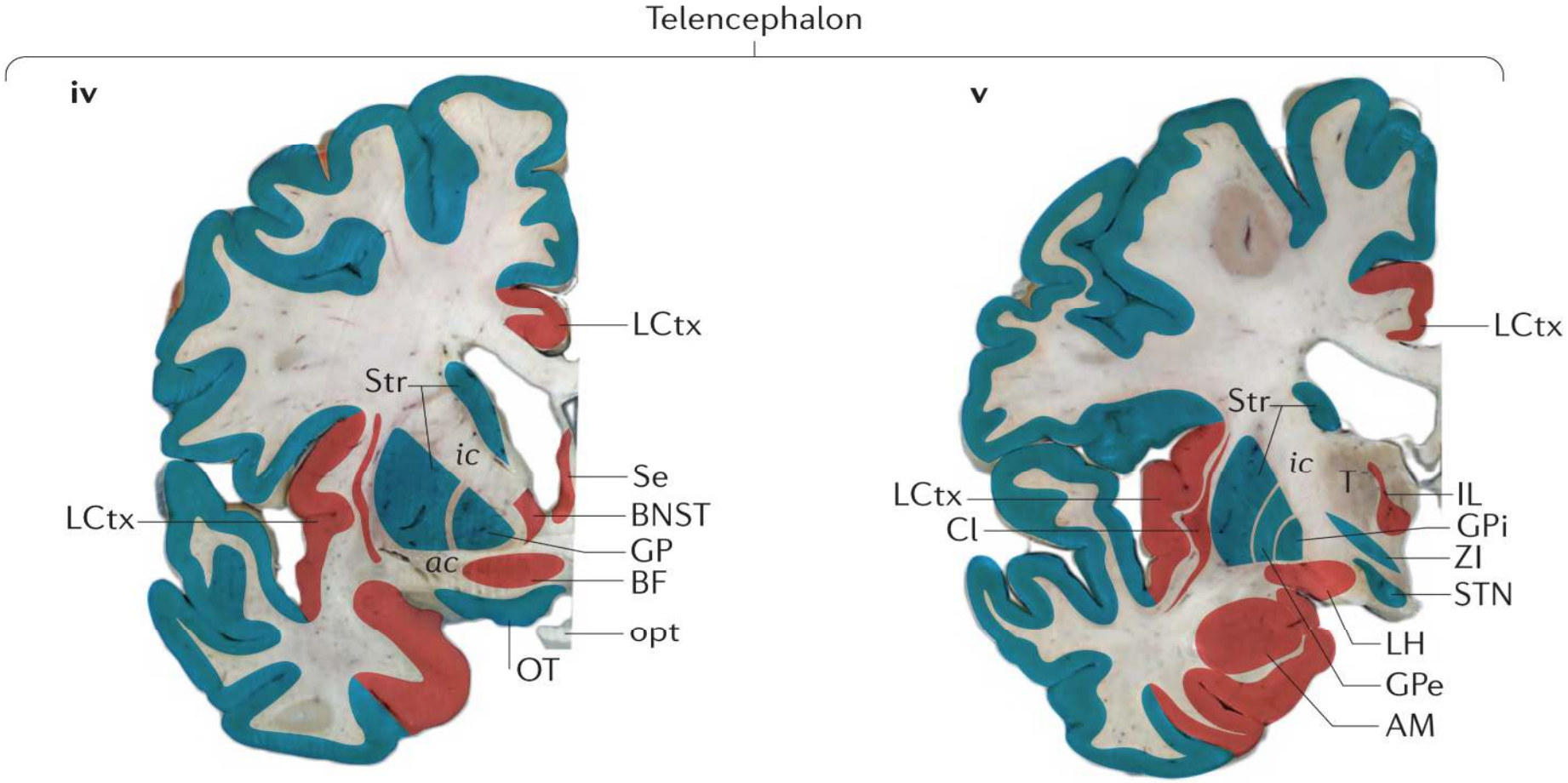
KEY: **3N** third cranial nerve (oculomotor) **cp**, cerebral peduncle; **R**, red nucleus; **SNd**, dorsal tier of the SNc; **SNv**, ventral tier of the SNc; **SNr** substantia nigra pars reticulata **SNc** substantia nigra pars compacta (DA projection system for neo-stratum); **VTA,** ventral tegmental area (DA projection system for paleostriatum and mesocortical systems).

Selective vulnerability of brainstem regions to Lewy pathology – indexing a hidden phenotype?

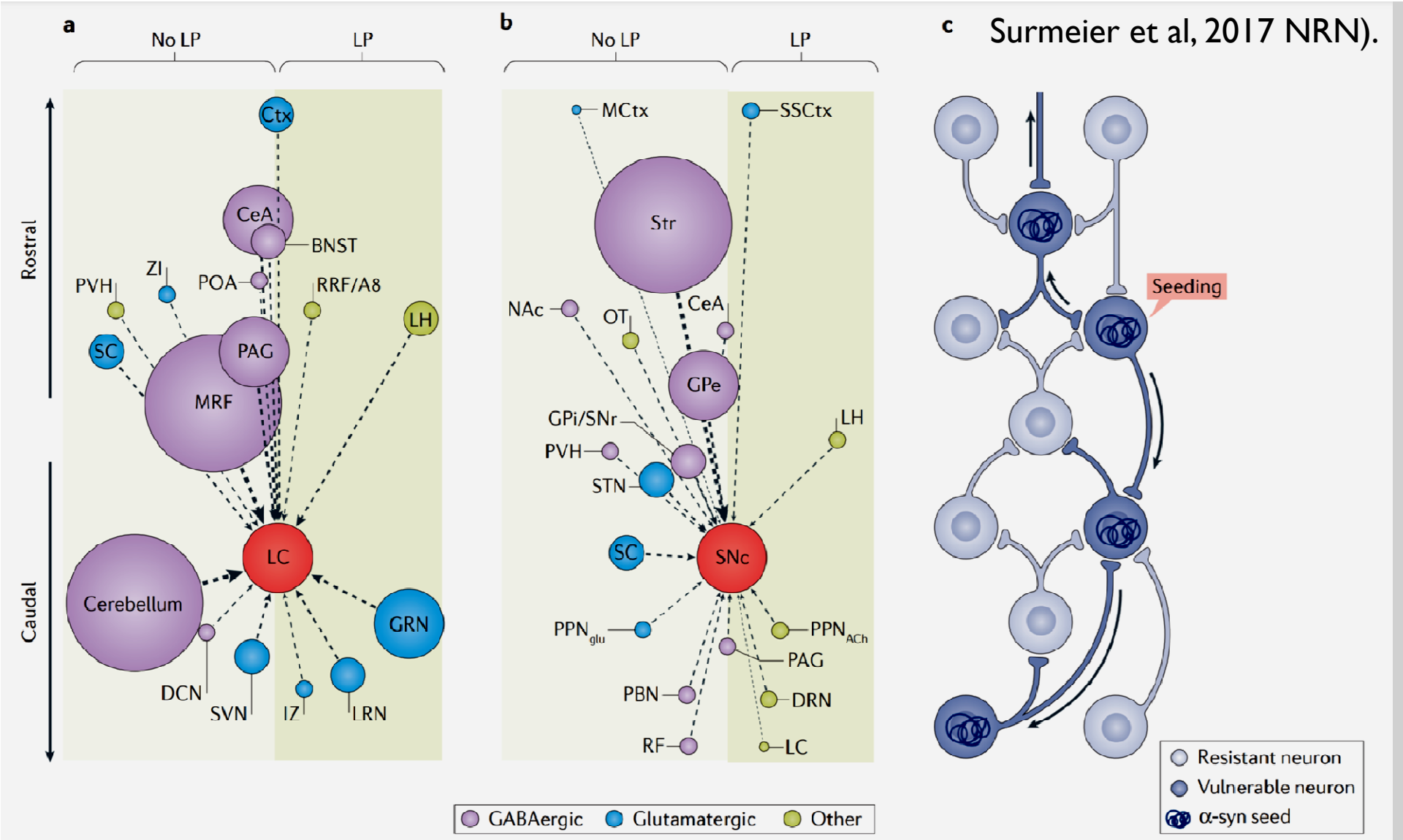


aq, aqueduct; **cp**, cerebral peduncle; **DMV,** dorsal motor nucleus of the vagus; **DRN**, dorsal raphe nucleus; **HN**, hypoglossal nucleus; **icp**, inferior cerebellar peduncle; **ion**, inferior olivary nucleus; **IZ**, intermediate reticular zone; **LC**, locus coeruleus; **mcp**, middle cerebellar peduncle; **MRN**, median raphe nucleus; **PAG,** periaqueductal grey; **PBN**, parabrachial nucleus; **PGRN/GRN**, paragigantocellular/gigantocellular reticular nucleus; PPN, pedunculopontine nucleus; **PRN**, pontine reticular nucleus; **pt,** pyramidal tract; **RM**, raphe magnus; **RRF/A8**, retrorubral fields/A8 dopaminergic cell group; **SC**, superior colliculus; **SNc**, substantia nigra pars compacta; **SNr,** substantia nigra pars reticulata; **SVN**, spinal vestibular nucleus; **VTA,** ventral tegmental area; ***(Surmeier et al, 2017 NRN).***

Similar selective vulnerability of forebrain?



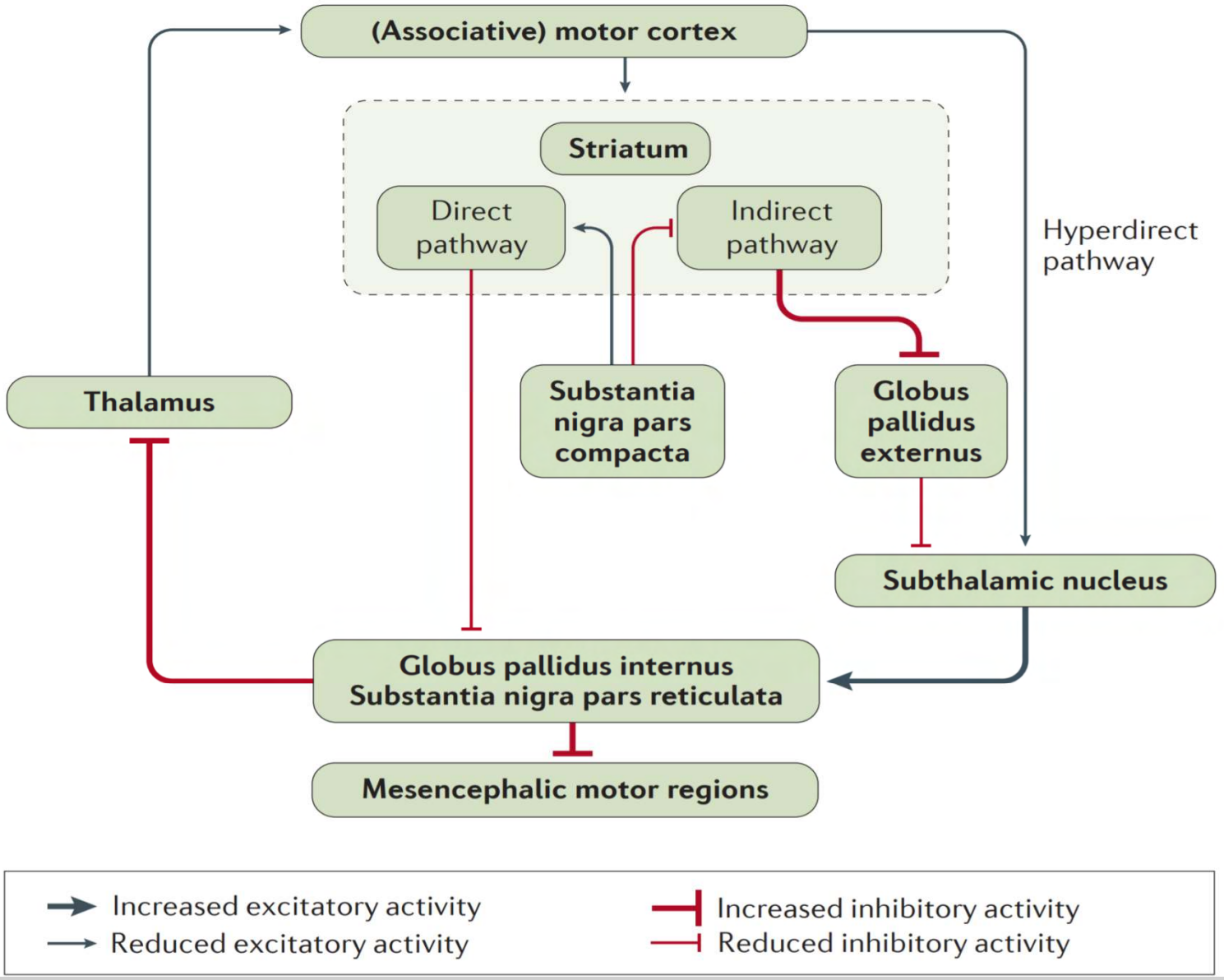
ac, anterior commissure; **AM**, amygdala; **BF,** basal forebrain; **BNST,** bed nucleus of stria terminalis; **Cl**, claustrum; **FCtx**, frontal cortex; **GP**, globus pallidus; **GPe**, GP externa; **GPi,** GP interna; **ic**, internal capsule; **IL**, intralaminar nuclei thalamus; **LCtx**, cingulate/insula; **LH**, lateral hypothalamus; **opt**, optic tract; **OT,** olfactory tubercle (limbic BG); **Se**, septum; **SNc**, substantia nigra pars compacta; **SNr,** substantia nigra pars reticulata; **STN**, subthalamic nucleus; **Str**, striatum; **T**, thalamus; **ZI**, zona incerta.



A plot of afferent connectome of mouse LC noradrenergic (**a**) & SNc DA (**b**) neurons.

These do not well predict pattern of postsynaptic, intraneuronal Lewy pathology observed in PD (assumes mouse & human connectomes are similar & that retrograde spread of α-synuclein dictated by number/strength of connections). Nuclei projecting to LC/SN represented as circles along a rostrocaudal vertical axis. Diameter of circle = strength of projection. Plot shows that strength of synaptic connection to LC is not correlated with LP!

Modeling effects of DA deafferentation of neostriatum on basal ganglia circuitry

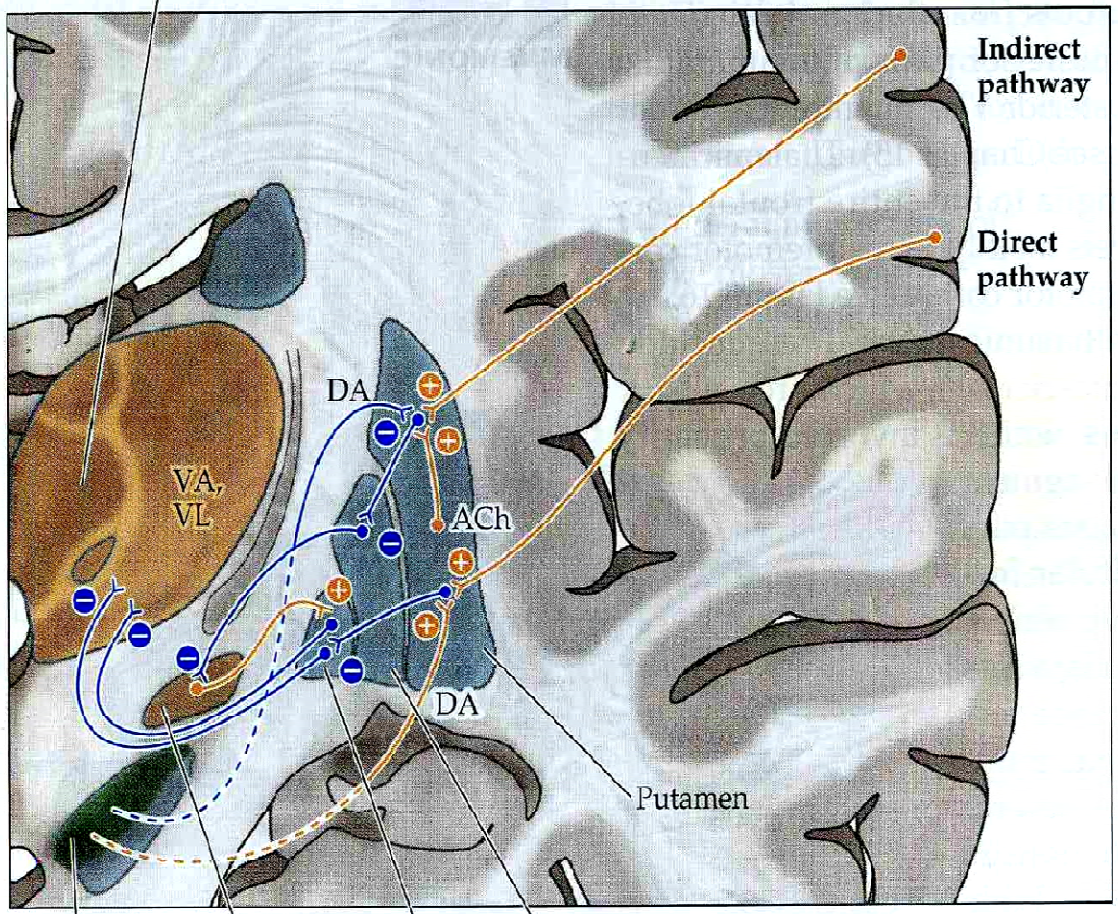


Classical models for PD, emphasizing direct & indirect pathway concepts. ***Direct pathway*** facilitates activation of cortex & mid-brain/cortical motor & premotor systems – allowing activation of habits; **indirect pathway** inhibits same. DA signals into these pathways promote activation of direct pathway, while inhibiting inhibitory indirect pathway. Net effect of dual DA loss is ***massive inhibition of habit & motor systems***.

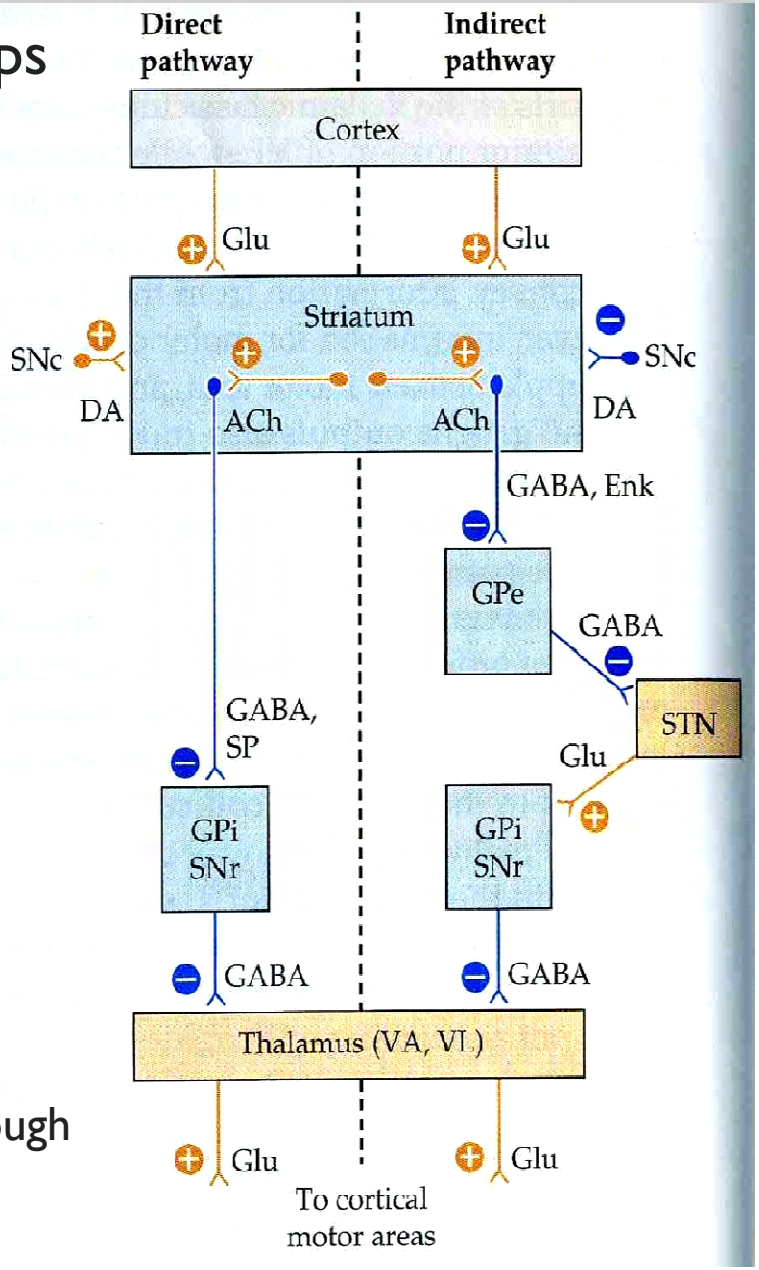
DA afferent loss putamen > caudate > NAcc

Basic Pathways: Direct and Indirect

Note: only shows basic motor loops



From Blumenfeld's Neuroanatomy Through Clinical Cases



Summary Review of PD Part I

- PD, like its first cousin AD, has both ~genetically determined & sporadic or somewhat genetically predisposed versions. MOTT genes + environs
- It's easy to confuse PD with Parkinsonian Plus disorders, less easy to miss the diffuse Lewy body variant which may be AD & PD.
- Pathogenic protein is α-synuclein – forming Lewy bodies at end point of aggregation. Fibrils may be worst/most degenerative assembly state.
- α-synuclein may be spread by connectivity (Braak's original idea) but connectivity alone does not determine pattern → vulnerable phenotype?
- Basal ganglia organize habits/skills, and nigral DA degeneration deprives both direct (DP) & indirect pathways (IP) of DA signal. Putamen>Caudate
- This ↓ 'gas pedal' of DP, ↑ brakes of IP – and since virtually all output of BG systems is inhibitory→ massive inhibition of both thalamocortical systems & ***midbrain locomotor nuclei***. Intrinsically depressing?!
- *What about genes, underlying neurobiology & mechanisms? And more neuroprotective treatment beyond conventional dopaminergic therapies?*

Genes, proteins and neurobiological and neuropathic mechanisms. Penetrating the vulnerable phenotype

Many of the same players mechanistically as Alzheimer's disease but with interesting network and histopathological differences.

PD predisposes to AD. Many AD patients have LBs.

AD and PD as very close first cousins?

Genetics of Parkinson's disease

- Heritable forms of Parkinson disease (fPD) only represent 5–10% of all cases but like in fAD provide clues about neural mechanisms.
- Some of these fPD (AD/AR) phenotypically/clinically indistinguishable from sPD, but others show distinctive/unusual LB patterns and disease phenotypes.
- Missense mutation of αSYN (PARK1) early onset, w/dementia. Duplication of αSYN (PARK4)→ onset in 50s: triplication → onset 30s w/ marked dementia.
- Mutations in 'vacuolar protein sorting-associated protein 35' (***VPS35***) → autosomal dominant PD, affects α-synuclein handling/trafficking to lysosome, Golgi apparatus, exosomes. LOF → ↓ proteostasis. Classic PD phenotype.
- ***LRRK2*** *G2019S* mutation: ↓ LAS w/ ↑aggregation of α-syn in DA neurons. Some mutations of LRRK2 don't show LBs, can have MSA, SND phenotypes.
- Most common genetic risk factor is heterozygous mutations in lysosomal enzyme ***GBA (***glucocerebrosidase), also reduce LAS function.
- GWAS show 2 polymorphisms in ***GBA*** associated w/ ↑risk of PD.
- Many genes also converge on MITO fxn and mitophagy. PINK is required for labeling/clearance of damaged MITO, as is PARKIN.
- Genes perturbed impact ***autophagy/proteostasis (LAS), α-synuclein, MITO function, Ca++ homeostasis, oxidative stress, inflammation***.

From Poewe et al, 2017 (NRDP)

Locus symbol	New designation‡	Gene locus	Gene	OMIM (phenotype MIM number; gene/ locus MIM number)	Clinical clues
Autosomal dominant Parkinson disease					
PARK1 or *PARK4*	PARK-*SNCA*	4q22.1	*SNCA*	• 168601; 163890 (*PARK1*) • 605543; 163890 (*PARK4*)	Missense mutations (*PARK1*) cause classic Parkinson disease phenotype. Duplication or triplication of this gene (*PARK4*) causes early-onset Parkinson disease with prominent dementia
PARK8	PARK-*LRRK2*	12q12	*LRRK2*	607060; 609007	Classic Parkinson disease phenotype. Variations in *LRRK2* include risk-conferring variants and disease-causing mutations
PARK17	PARK-*VPS35*	16q11.2	*VPS35*	614203; 601501	Classic Parkinson disease phenotype
Early-onset Parkinson disease (autosomal recessive inheritance)					
PARK2	PARK-*Parkin*	6q26	*PARK2* encoding parkin	600116; 602544	Often presents with lower limb dystonia
PARK6	PARK-*PINK1*	1p36.12	*PINK1*	605909; 608309	Psychiatric features are common
PARK7	PARK-*DJ1*	1p36.23	*PARK7* encoding protein deglycase DJ1	606324; 602533	Early-onset Parkinson disease
PARK19B	PARK-*DNAJC6*	1p31.3	*DNAJC6*	615528; 608375	Onset of parkinsonism between the third and fifth decades of life
Complex genetic forms (autosomal recessive inheritance)§					
PARK9	PARK-*ATP13A2*	1p36.13	*ATP13A2*	606693; 610513	Early-onset parkinsonism with a complex phenotype (for example, dystonia, supranuclear gaze palsy, pyramidal signs and cognitive dysfunction); also known as Kufor–Rakeb syndrome
PARK14	PARK-*PLA2G6*	22q13.1	*PLA2G6*	256600; 603604	PLAN (or NBIA2) is characterized by a complex clinical phenotype, which does not include parkinsonism in the majority of cases
PARK15	PARK-*FBXO7*	22q12.3	*FBXO7*	260300; 605648	Early-onset parkinsonism with pyramidal signs and a variable complex phenotype (for example, supranuclear gaze palsy, early postural instability, chorea and dystonia)
PARK19A	PARK-*DNAJC6*	1p31.3	*DNAJC6*	615528; 608375	Juvenile-onset parkinsonism that is occasionally associated with mental retardation and seizures
PARK20	PARK-*SYNJ1*	21q22.11	*SYNJ1*	615530; 604297	Patients may have seizures, cognitive decline, abnormal eye movements and dystonia
PARK23	Not yet assigned	15q22.2	*VPS13C*	616840; 608879	Young-adult-onset parkinsonism associated with progressive cognitive impairment that leads to dementia and dysautonomia

Monogenic variants association with PD

AD autosomal dominant
AR autosomal recessive

Table 1. Summary of monogenic variants associated with Parkinson's disease.

	Gene (HGNC Approved Name)	Alternative Gene Names	Inheritance	Pathogenicity	PD Phenotype	Function
High penetrance	*SNCA*	*PARK1, PARK4, NCAP*	AD	Pathogenic	Early-onset	Uncertain (encodes α-synuclein)
	VPS35	*PARK17, MEM3*	AD	Pathogenic	Typical	Retromer and endosomal trafficking
	PINK1	*PARK6*	AR	Pathogenic	Early-onset	Mitochondrial
	PARK7	*DJ-1*	AR	Pathogenic	Early-onset	
	PRKN	*PARK2, PARKIN*	AR	Pathogenic	Early-onset	
	PLA2G6	*PARK14, IPLA2*	AR	Pathogenic	Early-onset, atypical	Cell membrane
	ATP13A2	*PARK9*	AR	Pathogenic	Early-onset, atypical	Lysosomal
	FBXO7	*PARK15, FBX7*	AR	Pathogenic	Early-onset, atypical	Mitochondrial
	POLG	*POLG1, POLGA*	AD	Pathogenic	Early-onset, atypical	Mitochondrial DNA maintenance
	DNAJC6	*PARK19, DJC6*	AR	Likely pathogenic	Early-onset	Synaptic vesicle formation and trafficking
	DNAJC13	*PARK21, RME8*	AD	Conflicting reports	Typical	
	TMEM230	*C20ORF30*	AD	Conflicting reports	Typical	
	SYNJ1	*PARK20*	AR	Pathogenic	Early-onset, atypical	
	VPS13C	*PARK23*	AR	Pathogenic	Early-onset	Mitochondrial
	CHCHD2	-	AD	Pathogenic	Typical	Uncertain
	DCTN1	-	AD	Pathogenic	Atypical	Microtubule

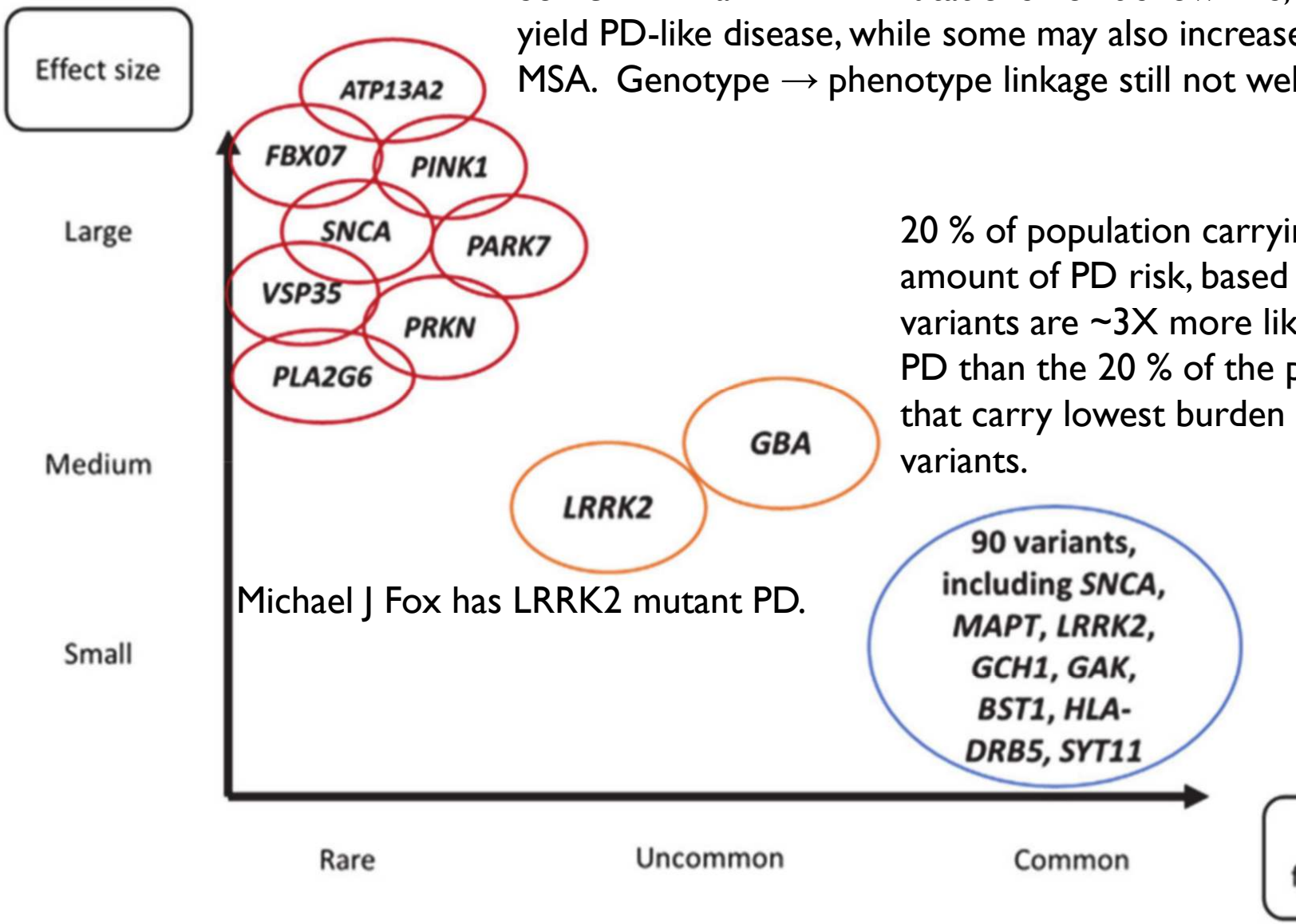
Variably penetrant and associated genes but not likely to be pathogenic genetic variants

Variable penetrance	*LRRK2*	*PARK8, DARDARIN*	AD	Pathogenic	Typical	Lysosomal, mitochondrial, microtubule
	GBA	*GBA1*	AD	Pathogenic	Typical	Lysosomal
Associated with PD but unlikely to be pathogenic	*HTRA2*	-	AD	Uncertain/likely benign	-	Mitochondrial
	UCHL1	*PARK5*	AD	Uncertain/likely benign	-	Ubiquitin-proteasome
	GIGYF2	*PARK11*	AD	Uncertain/likely benign	-	Uncertain
	EIF4G1	-	AD	Benign	-	mRNA translation
	LRP10	*LRP9*	AD [1]	Uncertain	-	Uncertain

Early twin studies showed only a slight excess of PD in monozygotic compared to dizygotic twins and led to a premature conclusion that PD was an exclusively acquired condition, but discovery of a first familial form of PD due to a single gene mutation and later twin studies incorporating a longitudinal design have led to the current paradigm of PD as a complex disease with both genetic and environmental contributions. Meta-analysis suggests that the presence of a family history of Parkinson's confers a 3–4x increase in PD risk, implying a significant effect of shared genetic and environmental factors to sporadic PD risk (Day & Mullin, 2021)

Mapping of genetics of PD – high risk or deterministic genes rare, low risk SNPs common.

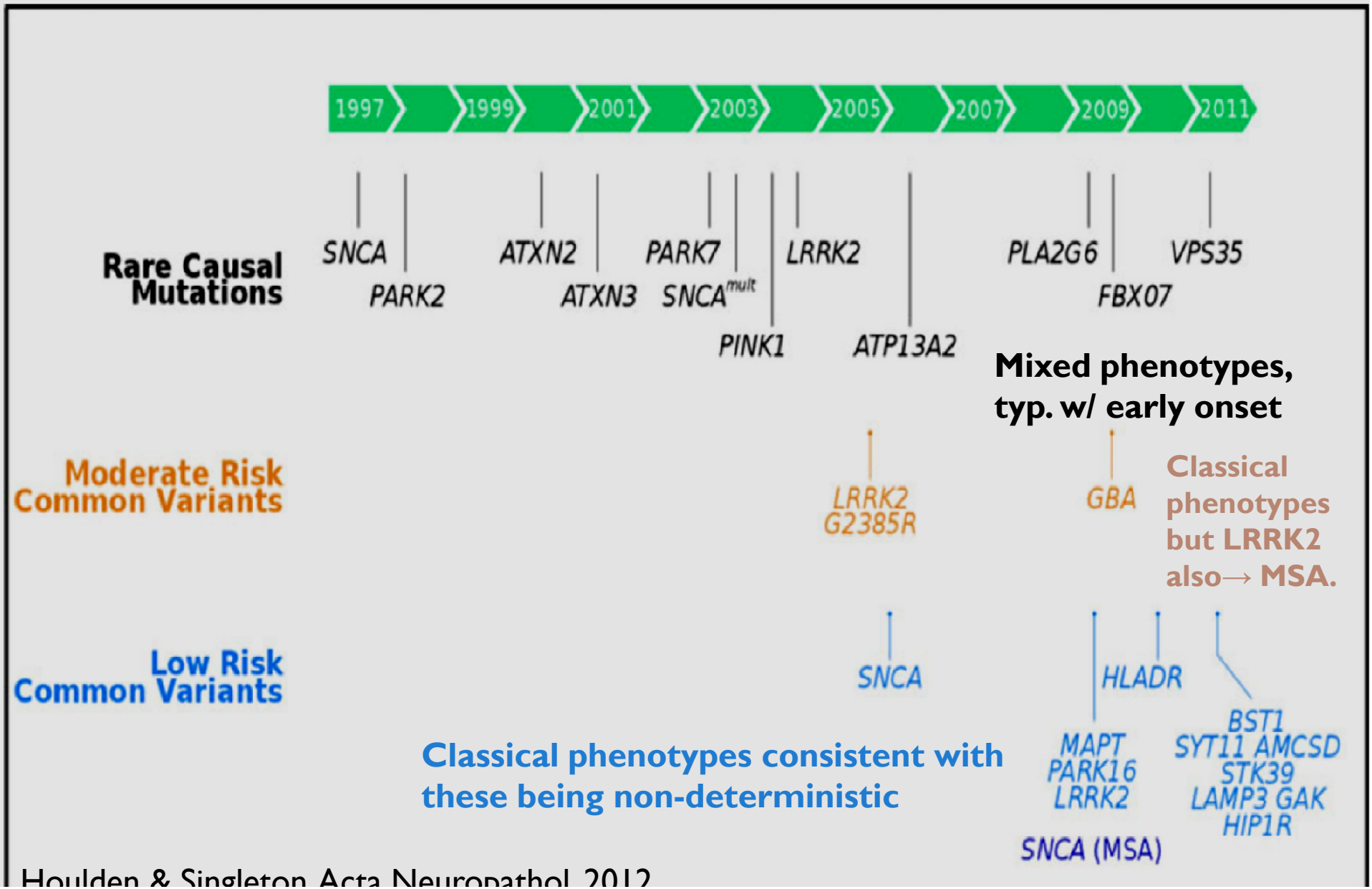
Some PARK and LRRK mutations don't show LBs, but still yield PD-like disease, while some may also increase risk for MSA. Genotype → phenotype linkage still not well mapped.



20 % of population carrying highest amount of PD risk, based on these 90 variants are ~3X more likely to have PD than the 20 % of the population that carry lowest burden of risk variants.

Michael J Fox has LRRK2 mutant PD.

Timeplot of discovery of risk genes/variants



Houlden & Singleton Acta Neuropathol 2012

Current Animal Models of PD

PD models	Type	Notes
Pharmacological	Reserpine	Induces transient parkinsonian symptoms without nigral dopaminergic neurodegeneration
	Haloperidol	Blocks striatal dopaminergic transmission
Environmental	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	Widespread animal models. Rapid neurodegeneration does not mirror the chronic and progressive evolution of PD
	6-hydroxydopamine (6-OHDA)	
	Rotenone	Less frequently used models. Induce dopaminergic neuron loss and Lewy bodies. The main limitations are peripheral toxicity and extent inflammation
	Paraquat	
	Isoquinolines	
	Methamphetamine	
	Proteasome inhibitor I (PSI)	Induce nigrostriatal neuron loss and Lewy bodies with progressive motor disability. High variability and low reproducibility.
	Epoximicin	
	Lipopolysaccharide	Induce inflammation and related nigrostriatal neuronal loss. Does not replicate PD features.
Genetic	Parkin	Knock-out mice for these genes show motor dysfunction, but no neurodegeneration
	PTEN-induced kinase 1 (PINK1)	**PINK is MITO depolarized membrane protein which signals for mitophagy, Parkin also required**
	Protein deglycase (DJ-1)	**DJ-1 also a MITO OS sensor and rescue protein**
	Leucine-rich repeat kinase (LRRK) 2	Knock-in mice show alteration of dopaminergic transmission, without clear behavioral deficits
	Vacuolar protein sorting-associated protein (VPS) 35	Knock-out mice show a reduction in striatal dopamine level, accompanied by α-Synuclein accumulation and motor deficits
	Mitopark	The specific loss of the gene coding for mitochondrial transcription factor A in dopaminergic neurons leads to a progressive decline of neuronal function and slow development of PD-related behavior
	α-Synuclein models	• Thy-α-Syn; • A53T α-Syn overexpression; • Aggregates of α-Syn pre-formed fibrils (PFF) Useful for the study of pathogenic mechanisms rather than for drug development.

The lack of full 'translatability' renders preclinical research of PD still a challenge. To date, animal models are divided into 3 main groups: pharmacological, environmental and genetic. Although each model incorporates some PD features, the development of new experimental models esp. combining gene and environs would contribute to ↑understanding of pathogenic mechanisms. Toxin models increasingly seen as poor approach to modeling progressive ND.

α-Synuclein Proteostasis and PD

- Alpha synuclein (140 AA) encoded by SNCA gene, where point mutations or multiplications of gene linked to heritable PD – polymorphisms that increase expression of gene also increase PD risk.
- Unclear what α-synuclein does (like Aβ?) but w/functional roles in neuroplasticity, synaptic vesicle fxn, MITO function, intracellular trafficking/chaperone molecule functions. Upregulated in aging, like Aβ.
- Similar aggregation story to β-amyloid. Monomers → oligomers → fibrils → large aggregates (Lewy bodies). HMW oligomers problematic, like AD, but fibrils more so? Number of LB poorly correlated with disease severity, like plaque in AD.
- α-synuclein oligomers may preferentially disrupt DA neurotransmission via several issues: DA terminal release, synaptic vesicle fxn, others issues. AβO ☹ ACh, Glut
- Degraded by proteasome, 'chaperoned' autophagy and by macro-autophagy systems. In neurons w/α-synuclein accumulation, lysosomal fxn degraded, autophagosomes accumulate, suggesting autophagy failure.
- Also like Aβ, α-synuclein inhibits both proteasome and macro-autophagy.
- All this suggests shared vulnerabilities in NDD? ***Declining proteostasis in relationship to proteins w/↑expression from neuroplasticity challenge of aging that simultaneously inhibit or challenge autophagy systems.***
- Thin margins to begin, creating ↑opportunity for homeostatic failure?

Spread of alpha synuclein pathology – similarities w/AβO/tau seeding/propagation. Cautions!

- Braak's original concept: prion-like propagation: α-syn aggregates in neuron, transported intra-axonally, released by exosomes, taken up by neighboring neurons seeding aggregation.
- 1st sites for α-synuclein aggregation: enteric nervous system, DMV & olfactory bulb → consistent with symptoms of prodromal PD (i.e., constipation & anosmia).
- As demonstrated in prior slides, seeding & aggregation related to unknown phenotypic vulnerabilities, **not** simple connectivity. ***Big caveat to the classic Braak model.***
- Cell culture studies: ↓ LAS manipulation → ↑ secretion of α-synuclein into extra-cellular space via exosomes, w/endocytosis into post synaptic cells. Suggests strong linkage between LAS issues & α-syn. *Fibrils > oligomers* might be candidates for prion-like activity.
- KO of α-SYN prevents MPTP DA toxicity, suggesting α-SYN forms a permissive factor, promoting Ca++ issues & MITO failure, but also pointing to extra α-SYN factors.
- BUT . . .(similar to AD), mAB aimed at α-synuclein successful in animal models only?
- Lots of other evidence – not attended to – that proteinopathy ≠ neurodegeneration.
  - Some 10-15% of elderly patients have significant LBs without motor or cognitive issues.
  - Degree of SN loss ***not proportional*** to either degree of LB in SN or classic Braak staging (!)
  - LB pathology is ***not even necessary*** for SN degeneration in PD (eg, LRRK2/PARK mutations)
  - PD shows – just like AD – ***multiple proteinopathies*** esp. in older cohorts. **WUWT???**
  - Also just like amyloid family in AD, α-syn may be both compensatory neuroplasticity protein and an immune-related anti-pathogen protein, considerably complicating interpretation.

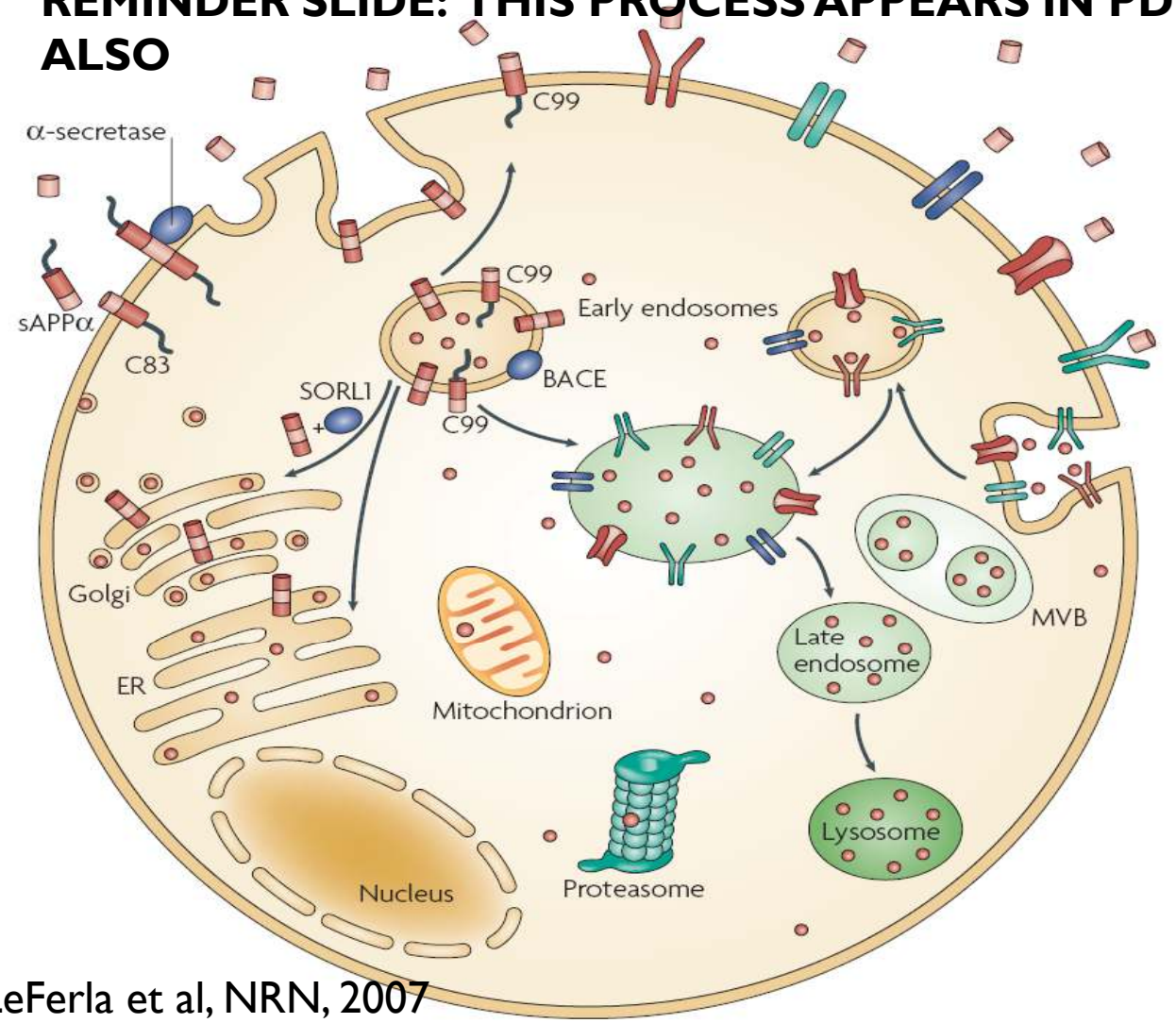
Beta Amyloid oligomers **interact with rAGE, lipid, NMDA, and nicotinic receptors**. APOE4 facilitates intake of Aβ/other proteins more than other alleles. Intracellularly, Aβ42+ interferes w/ MITO, proteasome, lysosome/ autophagy.

APOE4/Aβ42 negatively impact autophagy (which ↓ toxic proteins). Binding to **rAGE** increases inflammation/glial activation.

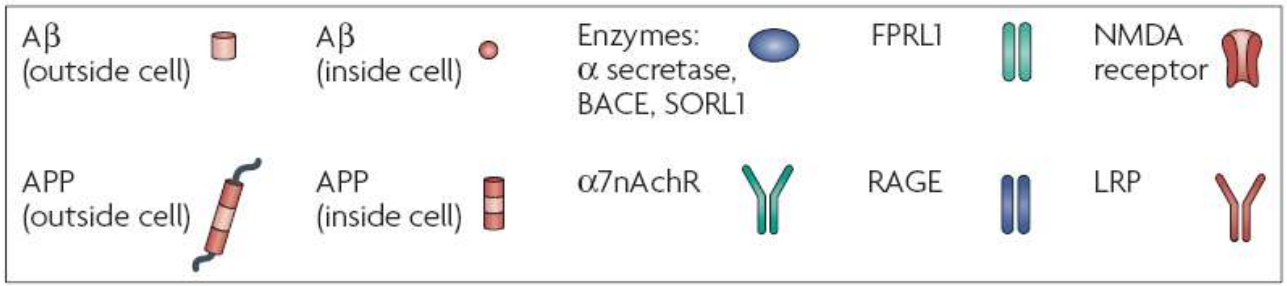
Aβ assembly state is critical to its pathogenic effects – oligomers more destructive re: many cellular functions. These effects may impact many different types of cells (i.e. pericytes,glia, oligo-dendrocytes - not just neurons)

α7nAChR, α7 nicotinic-ACh receptor; FPRL1, innate immune receptor; LRP, LDL receptor; MVB, multivesicular body; NMDA, N-methyl-d-aspartate; RAGE, receptor for advanced glycation end products

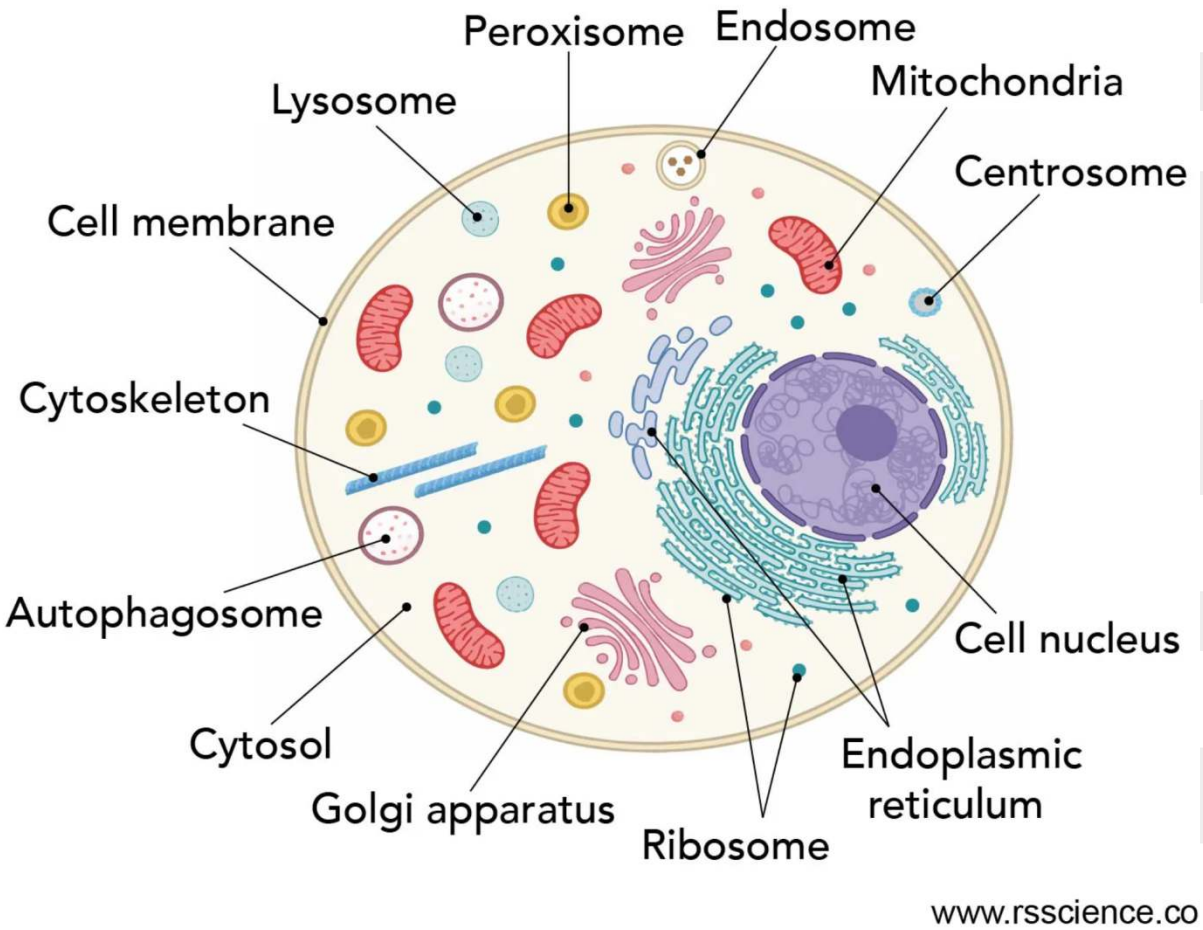
REMINDER SLIDE: THIS PROCESS APPEARS IN PD ALSO



LeFerla et al, NRN, 2007



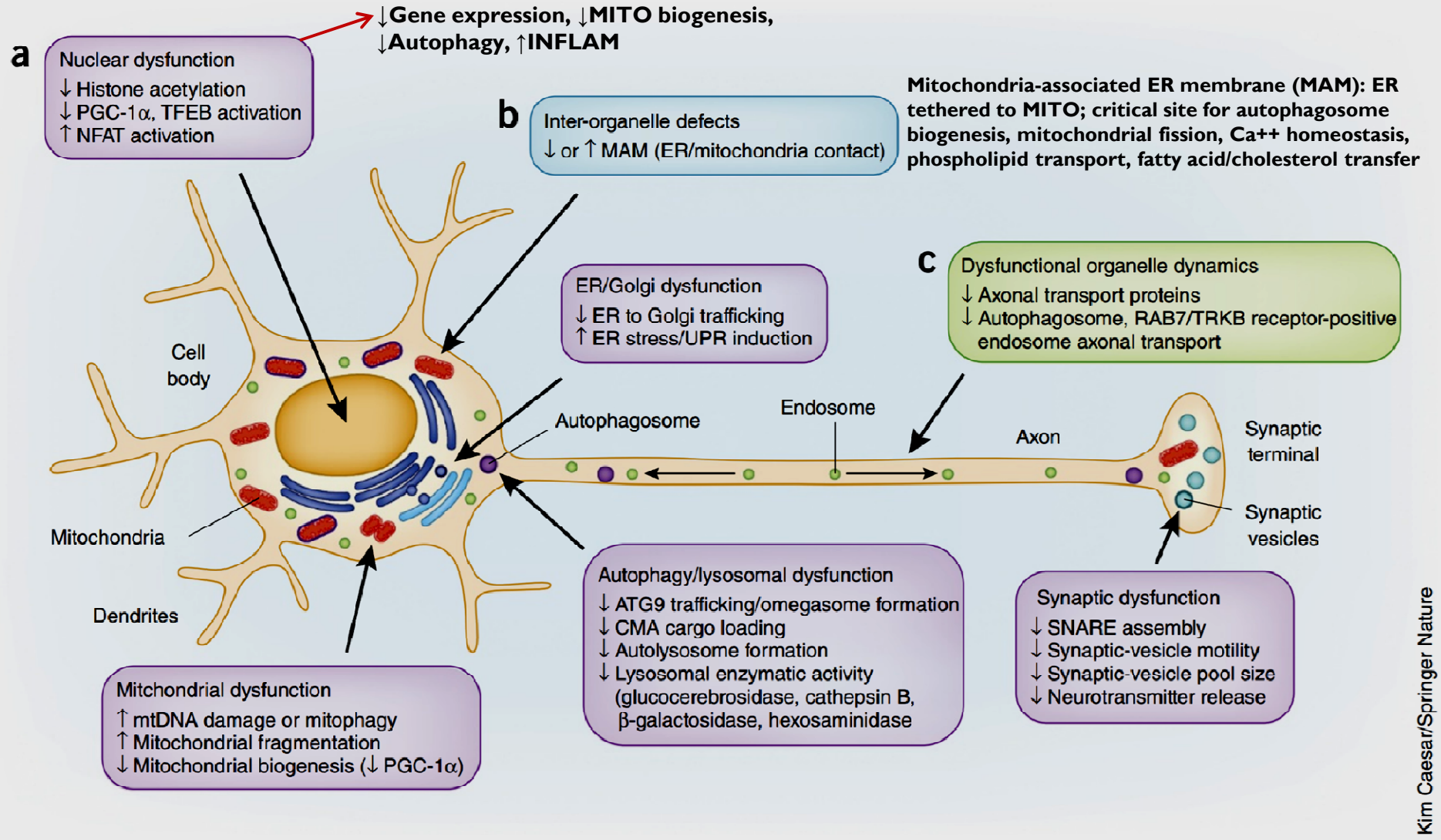
Eukaryotic Cellular Organelles Functions



Cell Organelle	Biological Function	Factory Part
Nucleus	DNA Storage	Files and blueprints management
Mitochondrion	Energy production	Powerplant
Ribosome	Protein synthesis	Machine to product toys
Rough ER	Protein production and modification	Coordination of toy production line and decoration
Smooth ER	Lipid production and Detoxification	Accessory production
Golgi apparatus	Protein transportation and export	Packaging and shipping department
Peroxisome	Lipid breakdown; redox reactions	Hazard chemical handling
Lysosome	Protein destruction	Recycling
Cytoskeleton	Cell movement; intracellular transportation	Conveyor system
Cell membrane	Define the inside and outside of a cell	Factory building
Cell wall	Structural support and protection (plant cell)	Reinforced factory building
Cytosol	Cellular fluid	Internal space and floor plan
Chloroplast	Photosynthesis (plant cell)	Solar panels
Vacuole	Storage and water regulation (plant cell)	Storage spaces

FYI brief refresher for those not familiar with cellular organelle concepts!

Multiple cellular organelles/fxns impacted by (too much?) α-synuclein – basic findings (Wong & Krainc, 2016 Nature Medicine)



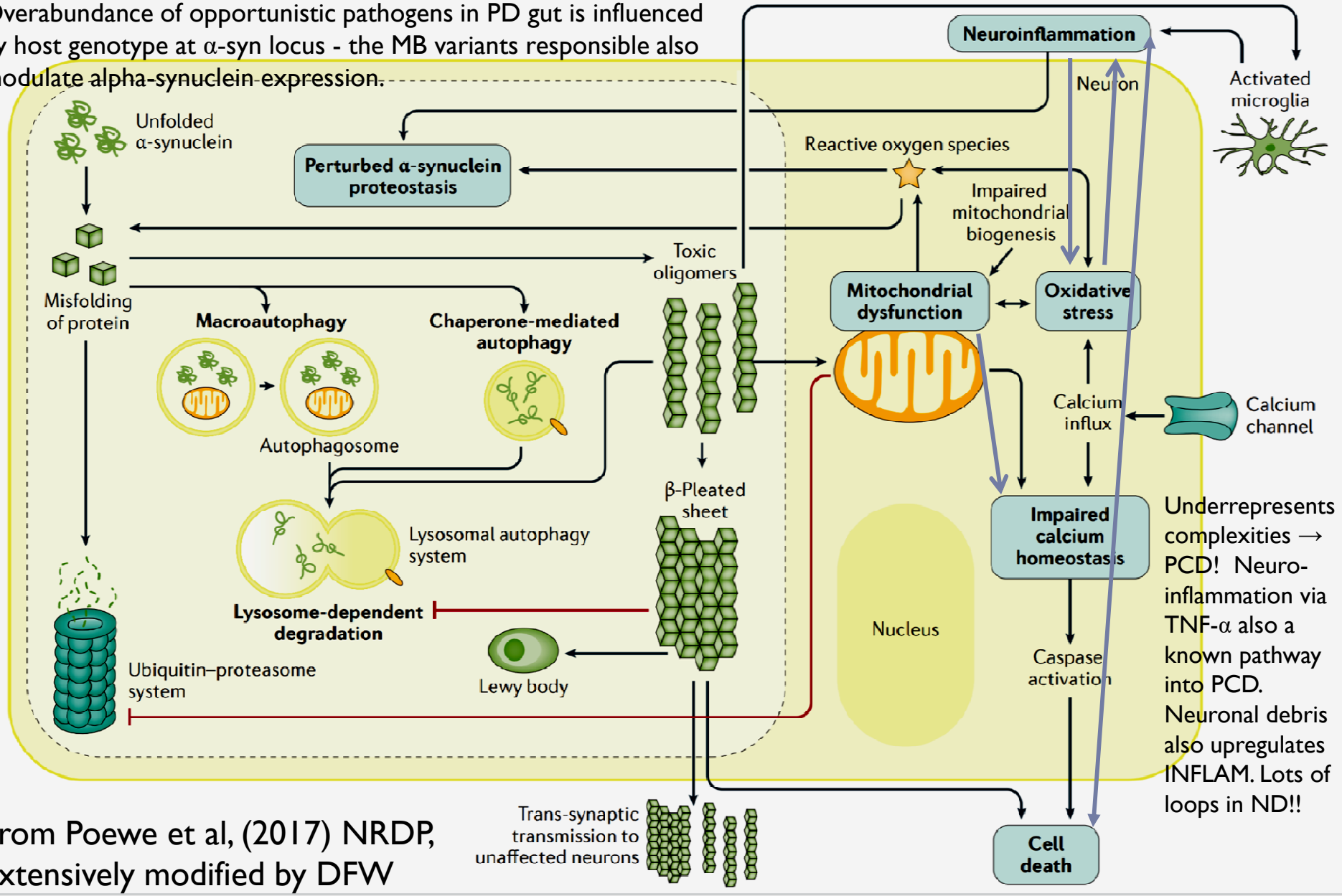
Familiar set of players in NDD? ↓PGC-1a, ↓TFEB/flux in autophagy systems, ↑synaptic dysfxn, jammed up transport/communication along axons, etc. α-SYN→ ↓DA release, ↓DAT.

↓ Proteostasis ↔ ↓ Mitochondrial function ↔ Axonal function. All 3 potentiate apoptosis.

- α-synuclein aggregation and MITO dysfxn may be mutually promoting.
- Accumulation of α-SYN inside MITO → ↑OS.
- Activity of mitochondrial complex I, PPAR-γ/co-activator PGC-1α (critical for MITO biogenesis) all shown reduced in PD.
- In vitro expression of PGC-1α reduces α-SNC, whereas knockdown of PGC-1α increases vulnerability to α-synO in animal models.
- Oligomers of α-syn in turn reduce expression of PGC-1α.
- Various suppressive/disruptive manipulations of mitochondrial genes (↓ ETC) in animal models → Parkinsonian phenotypes.
- Axonal function is esp. energically demanding, w/axonal degeneration a known feature of several MITO disease.
- Axons degenerate in substantial nigral neurons *before* neurons die.
- Consistent with this, α-SYN aggregates block axonal transport (~AD?)
- LRRK2, PARK, and PINK mutations all contribute to ↓ clearance of dysfunctional mitochondria (↓'mitophagy') & combined w/ suppression of PGC-1α, these mutations create energic deficits/failure.
- Mitochondrial failure appears a primary pathway into PCD and PD.

Neuropathic-mechanistic loops show many overlapping players with AD pathogenesis – also quite recursive!

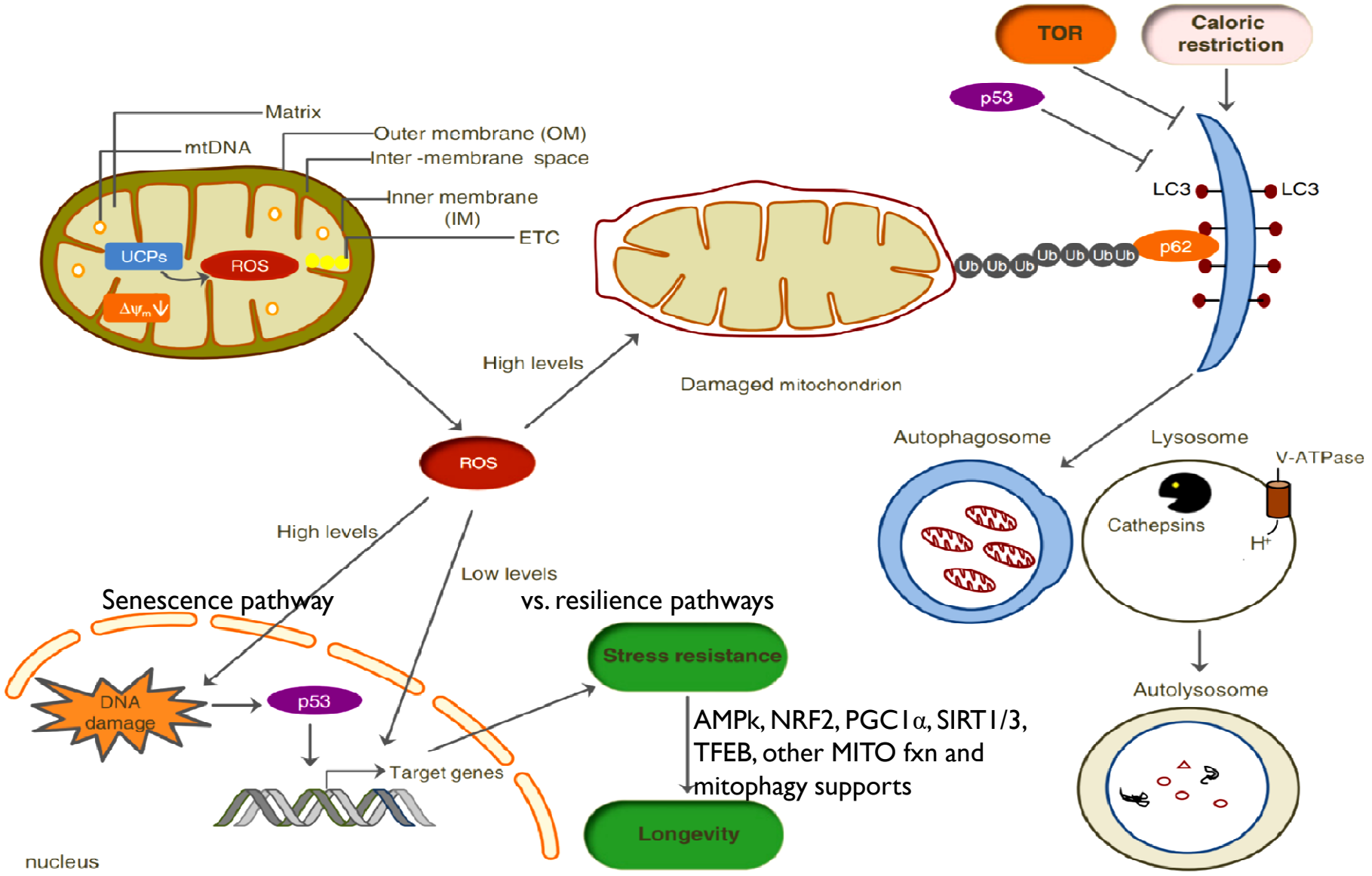
Overabundance of opportunistic pathogens in PD gut is influenced by host genotype at α-syn locus - the MB variants responsible also modulate alpha-synuclein expression.



Underrepresents complexities → PCD! Neuro-inflammation via TNF-α also a known pathway into PCD. Neuronal debris also upregulates INFLAM. Lots of loops in ND!!

From Poewe et al, (2017) NRDP, extensively modified by DFW

Factors promoting MITO resilience & mitophagy vs MITO failure, apoptosis and senescence



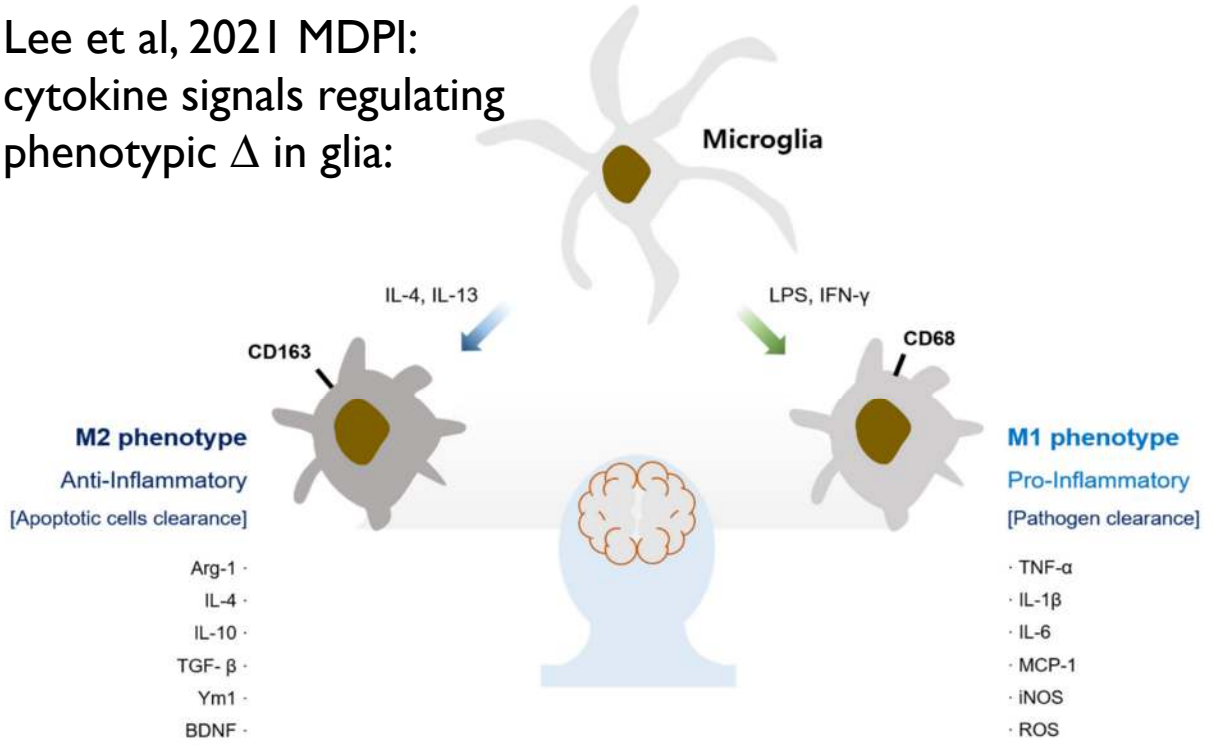
Where is astroglial system in Parkinson's disease? Still unclear . . . ?

- Knockout of INF β → Parkinsonian phenotype w/synucleinopathy – suggesting that virally-directed subset of INFLAM is neuroprotective of SN DA system. How????
- α-syn acts as agonist at TLR2/4 (toll like receptor2/4) → microglial activation.
- Microglial activation in both MSA & PD, (FrontalCtx/pons/midbrain/putamen) suggesting SN degeneration has contribution from INFLAM in α-syn diseases, but MGA ≠ correlate with symptom severity or [^{18}F]-dopa PET imaging of DA deprivation of basal ganglia ????
- Astrocyte phenotype change also seen (lower left).

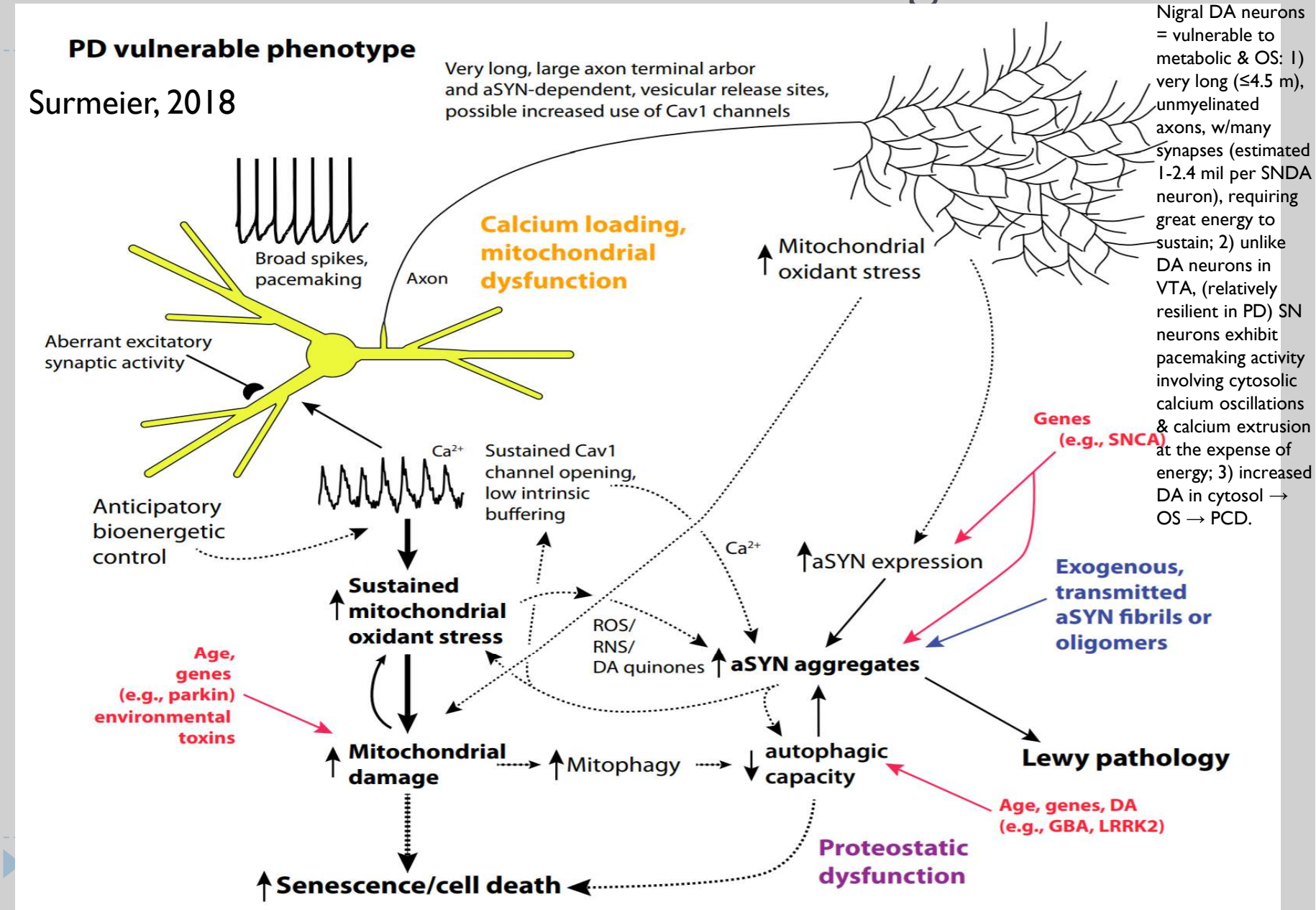
Astrocytes in PD:

1) DA → Ca^{++} astrocyte regulation: DA loss→ PCD (+ feedback in NDD)
2) ↑expression of α-syn in astrocytes from cPD pts.
3) Astrocyte-derived GMF → ↑ NF-κB/GM-CSF → ↑ M1 phenotype in MG → PCD
4) Anti-INFLAM interventions protective in AM, phase II trials of GLP1 agonist. PIII?

Lee et al, 2021 MDPI: cytokine signals regulating phenotypic Δ in glia:



Why are nigral and not ventral tegmental DA fields more vulnerable to neurodegeneration?

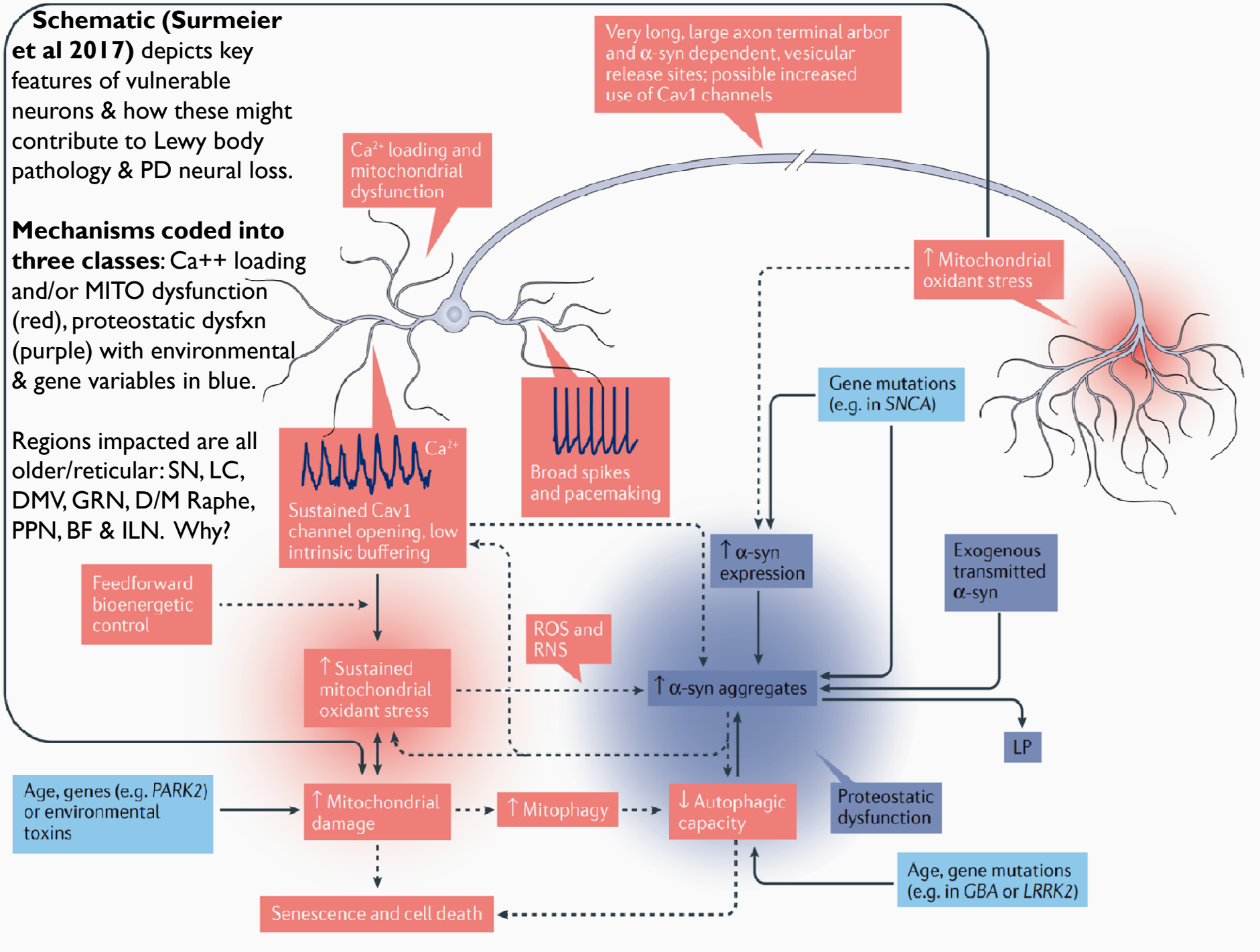


Nigral DA neurons = vulnerable to metabolic & OS: 1) very long (≤4.5 m), unmyelinated axons, w/many synapses (estimated 1-2.4 mil per SNDA neuron), requiring great energy to sustain; 2) unlike DA neurons in VTA, (relatively resilient in PD) SN neurons exhibit pacemaking activity involving cytosolic calcium oscillations & calcium extrusion at the expense of energy; 3) increased DA in cytosol → OS → PCD.

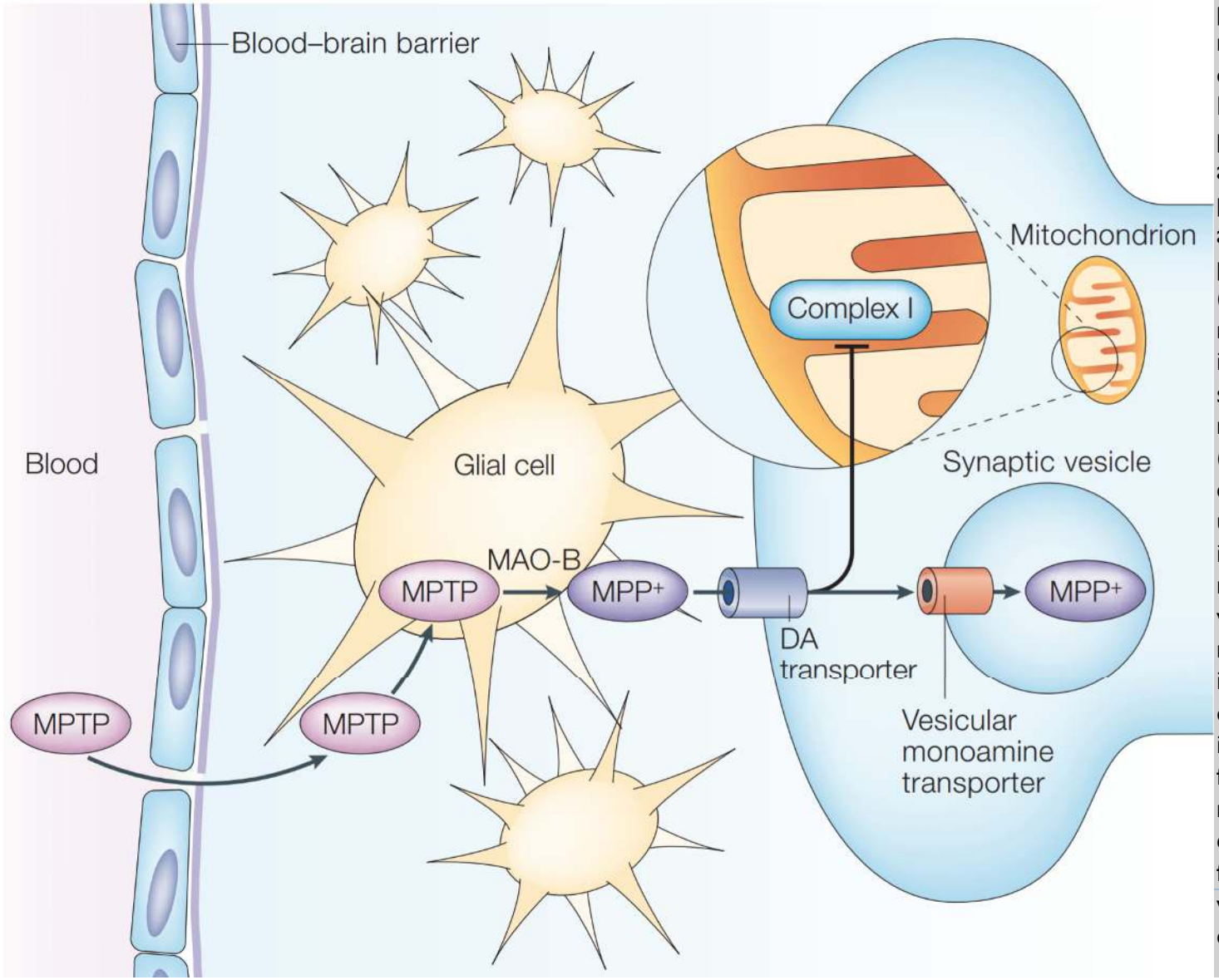
Schematic (Surmeier et al 2017) depicts key features of vulnerable neurons & how these might contribute to Lewy body pathology & PD neural loss.

Mechanisms coded into three classes: Ca++ loading and/or MITO dysfunction (red), proteostatic dysfxn (purple) with environmental & gene variables in blue.

Regions impacted are all older/reticular: SN, LC, DMV, GRN, D/M Raphe, PPN, BF & ILN. Why?



MPTP toxicity mediated by MITO complex 1 blockade



MPTP causes damage to the nigrostriatal dopamine (DA) dopamine pathway identical to PD, w/ exception of intraneuronal inclusions (Lewy bodies). Post mortem brain samples from PD pts show selective defects in mitochondrial electron transport chain complex. Metabolism of MPTP is complex, multistep process. After its systemic administration, MPTP, which is a pro-toxin, rapidly crosses BBB and is metabolized to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+) by the enzyme monoamine oxidase B (MAO-B) in non-DA cells, and then, via spontaneous oxidation, to 1-methyl-4-phenylpyridinium (MPP+), the active toxic compound. MPP+ is then taken up by DA transporters, for which it has high affinity. Once in DA neurons, MPP+ is concentrated within MITO, where it impairs mitochondrial respiration by inhibiting complex I of the electron transport chain. The inhibition of complex I impedes flow of electrons along mitochondrial electron transport chain→ increased production of free radicals (oxidative stress) with activation of programmed cell death molecular pathways.

Neuroinflammation ↔ OS ↔ aSYNopathy → PD

- Mitochondrial dysfunction, oxidative stress, and failure of autophagy are all pro-inflammatory. INFLAM ↔ OS, and both ↑ aSYN pathology.
- Classic Parkinsonian genes i.e. LRRK2 Leucine-rich repeat kinase 2 function at intersection of many systems, regulating microglial function (and impacting host of pro-neuroplastic operations – neurite outgrowth, cytoskeletal maintenance, vesicle trafficking, autophagy).
- In animal model (Gao et al, 2011), LPS injection →indistinguishable acute neuroinflammation initially in both WT & Tg SNCA mice; but only Tg mice → persistent neuroinflammation, chronic/progressive degeneration of SN DA pathways, accumulation of aggregated, nitrated α-syn, & formation of Lewy bodies in SNpc neurons.
- Gao et al, (2011), showed that 4-week infusion w/inhibitors of iNOS and NADPH oxidase, free radical-generating enzymes in activated microglia, blocked nigral α-syn/neurodegeneration in LPS-injected Tg mice.
- Suggests that (nitrox)oxidative stress generated in activated microglial systems critical to synucleinopathy and SN DA degeneration in PD.

Similarities and differences across AD/PD

- Both show ~deterministic and risk genes, with familial forms rarer.
- Both show a variable age of onset but sporadic PD ~50s, AD ~70s.
- Both classically approached as proteinopathies with pathogenesis attributed principally to protein (focus on issues beyond putative protein toxicity has significantly expanded recently.
- AD shows dual proteinopathy (Aβ, tau) sometimes more w/LBs or TAR DBP), PD sometimes also shows this (LBs plus AD pathology).
- Both show autophagy failure – all pathognomonic/pathogenic proteins (α-syn, Aβ, tau) intrinsically difficult to clear.
- Do multiple proteinopathic manifestations happen serially?
- Both show evidence for ↓ mitochondrial function, esp. in PD.
- Both show evidence for inflammation/activated microglia, esp. in AD.
- Synaptic depression (shared) from oligomers outside cell more in AD, more synaptic depression coming from α-syn inside cell in PD?

Current Treatment Paradigms

Treatments in the Pipeline with Neuroprotective/Disease-Modifying Effects

Similar to AD story with many treatments efficacious in animal models, not so much so clinical disease.

Current clinical paradigms – diagnosis, biomarkers, order of therapies & problems

- L-Dopa still standard of care, although sometimes DA agonists used 1st. Dx instituted at onset of Cardinal Triad – typically w/laterality, esp. of resting tremor.
- Occasionally non-cognitive symptoms like REM SBD, early autonomic sxn→ Dx.
- Biomarkers rarely obtained – expensive. Fluorine labeled L-dopa, now replaced by SPECT, several MRI diffusion series, myocardial sympathetic denervation.
- Surprisingly, no clinically useful CSF-based or serum diagnostic markers (!)
- Genetic assays informative (~2-3% of PD) if ↑family history or presenting with early-onset or dystonia (not typically seen in sPD). Finding a genetic locus currently ≠ change in Rx algorithms, but might in the future.
- In the pipeline, digital assay of movement may soon detect prodromal PD.
- Titration of L-dopa/DA agonists principally/exclusively to motor dysfunction.
- Because caudate is less DA deafferented than putamen, caudate loops overdriven, → additional overlay of cognitive disruption (rarely appreciated).
- Cognition rarely assessed in early-stage PD patients under meme that this is just a movement disorder, especially early on. Also, mood and sleep issues neglected.

MDS EBM Summary, 2018

TABLE 2. Conclusions on dopamine agonists (presented in alphabetical order)

Dopamine agonists		Prevention/delay of clinical progression	Symptomatic monotherapy	Symptomatic adjunct to levodopa	Prevention/delay of motor complications	Treatment of motor complications
Nonergot dopamine agonists						
Piribedil	Efficacy	Insufficient evidence	*Efficacious*	Efficacious	Insufficient evidence (F, D)	Insufficient evidence (F, D)
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	Investigational	*Clinically useful*	Clinically useful	Investigational (F, D)	Investigational (F, D)
Pramipexole	Efficacy	Insufficient evidence	Efficacious	Efficacious	*Efficacious (F, D)*	Efficacious (F) Insufficient evidence (D)
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	Investigational	Clinically useful	Clinically useful	*Clinically useful (F, D)*	Clinically Useful (F)
Pramipexole extended release	Efficacy	*Insufficient evidence*	*Efficacious*	*Insufficient evidence*	*Insufficient evidence (F, D)*	*Insufficient evidence (F, D)*
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	*Investigational*	*Clinically useful*	*Investigational*	*Investigational (F, D)*	*Investigational (F, D)*
Ropinirole	Efficacy	Insufficient evidence	*Efficacious*	*Efficacious*	Insufficient evidence (F) Efficacious (D)	Efficacious (F) Insufficient evidence (D)
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	Investigational	*Clinically useful*	*Clinically useful*	Investigational (F) Clinically useful (D)	Clinically useful (F) Investigational (D)
Ropinirole prolonged release	Efficacy	*Insufficient evidence*	*Likely efficacious*	*Efficacious*	*Insufficient evidence (F)* *Efficacious (D)*	*Efficacious (F)* *Insufficient evidence (D)*
	Safety	*Acceptable risk without specialized monitoring*				
	Practice implications	*Investigational*	*Possibly useful*	*Clinically useful*	*Investigational (F)* *Clinically useful (D)*	*Clinically useful (F)* *Investigational (D)*
Rotigotine	Efficacy	*Insufficient evidence*	*Efficacious*	*Efficacious*	*Insufficient evidence (F, D)*	*Efficacious (F)* *Insufficient evidence (D)*
	Safety	*Acceptable risk without specialized monitoring*				
	Practice implications	*Investigational*	*Clinically useful*	*Clinically useful*	*Investigational (F, D)*	*Clinically useful (F)* *Investigational (D)*

More on dopamine agonists, including parenteral

Parenteral nonergot dopamine agonist						
Apomorphine	Efficacy	Insufficient evidence	Insufficient evidence	Efficacious	Insufficient evidence (F, D)	Efficacious (F) Insufficient evidence (D)
	Safety	Acceptable risk without specialized monitoring when used as parenteral therapy.				
	Practice implications	Investigational	Investigational	Clinically useful	Investigational (F, D)	Clinically useful (F) Investigational (D)
Ergot dopamine agonists						
Bromocriptine	Efficacy	Insufficient evidence	Likely efficacious	Efficacious	Insufficient evidence (F) Likely efficacious (D)	Likely efficacious (F) Insufficient evidence (D)
	Safety	*Acceptable risk with specialized monitoring*				
	Practice implications	Investigational	Possibly useful	Clinically useful	Investigational (F) Possibly useful (D)	Possibly useful (F) Investigational (D)
Cabergoline	Efficacy	Insufficient evidence	*Efficacious*	Efficacious	*Efficacious (F, D)*	Likely efficacious (F) Insufficient evidence (D)
	Safety	*Acceptable risk with specialized monitoring*				
	Practice implications	Investigational	*Clinically useful*	Clinically useful	*Clinically useful (F, D)*	Possibly useful (F) Investigational (D)
Dihydroergocryptine	Efficacy	Insufficient evidence	Efficacious	Insufficient evidence	Insufficient evidence (F, D)	Insufficient evidence (F, D)
	Safety	*Acceptable risk with specialized monitoring*				
	Practice implications	Investigational	Clinically useful	Investigational	Investigational (F, D)	Investigational (F, D)
Lisuride	Efficacy	Insufficient evidence	Likely efficacious	Likely efficacious	Insufficient evidence (F, D)	Insufficient evidence (F, D)
	Safety	*Acceptable risk with specialized monitoring*				
	Practice implications	Investigational	Possibly useful	Possibly useful	Investigational (F, D)	Investigational (F, D)
Pergolide	Efficacy	*Unlikely efficacious*	Efficacious	Efficacious	*Insufficient evidence (F)* *Likely efficacious (D)*	Efficacious (F) Insufficient evidence (D)
	Safety	*Acceptable risk with specialized monitoring*				
	Practice implications	*Unlikely useful*	Clinically useful	Clinically useful	*Investigational (F)* *Possibly useful (D)*	Clinically useful (F) Investigational (D)

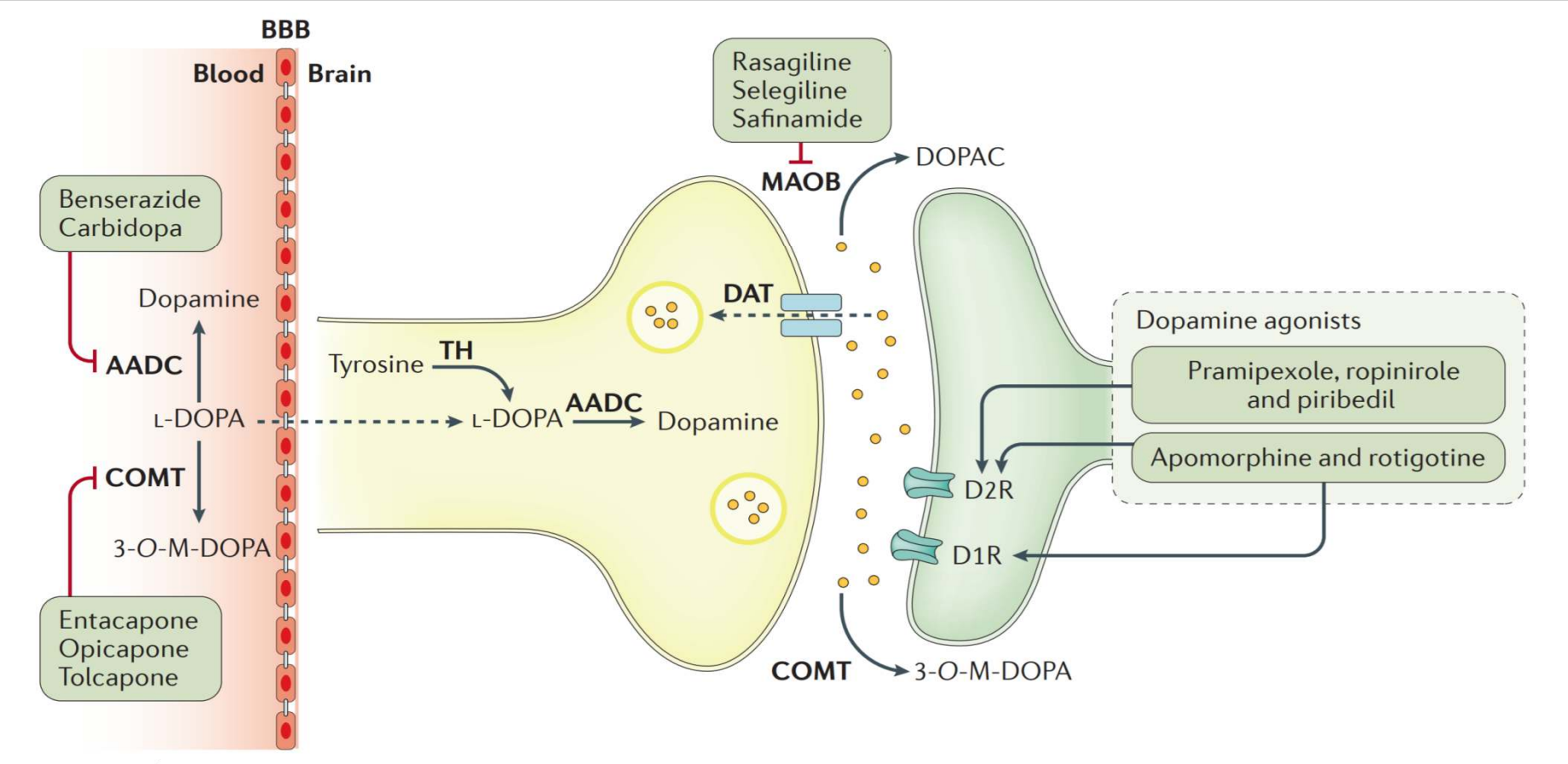
TABLE 3. Conclusions on levodopa

Levodopa		Prevention/delay of clinical progression	Symptomatic monotherapy	Symptomatic adjunct to levodopa	Prevention/delay of motor complications	Treatment of motor complications
Standard formulation	Efficacy	Insufficient evidence	Efficacious	N/A	Nonefficacious (F, D)	Efficacious (F) Insufficient evidence (D)
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	Investigational	Clinically useful	N/A	Not useful (F, D)	Clinically useful (F) investigational(D)
Controlled-release formulation	Efficacy	Insufficient evidence	Efficacious	N/A	Nonefficacious (F, D)	Insufficient evidence (F, D)
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	Investigational	Clinically useful	N/A	Not useful (F, D)	Investigational (F, D)
Rapid-onset oral formulation	Efficacy	*Insufficient evidence*	*Insufficient evidence*	*Insufficient evidence*	*Insufficient evidence (F, D)*	*Insufficient evidence (F)* *Insufficient evidence (D)*
	Safety	*Acceptable risk without specialized monitoring*				
	Practice implications	*Investigational*	*Investigational*	*Investigational*	*Investigational (F, D)*	*Investigational (F)* *Investigational (D)*
Infusion formulations	Efficacy	*Insufficient evidence*	*Insufficient evidence*	*Insufficient evidence*	*Insufficient evidence (F, D)*	*Likely efficacious (F, D)*
	Safety	*Acceptable risk without specialized monitoring*				
	Practice implications	*Investigational*	*Investigational*	*Investigational*	*Investigational (F, D)*	*Investigational (F, D)*

TABLE 4. Conclusions on COMT inhibitors

COMT inhibitors		Prevention/delay of clinical progression	Symptomatic monotherapy	Symptomatic adjunct to levodopa	Prevention/delay of motor complications	Treatment of motor complications
Entacapone	Efficacy	Insufficient evidence	N/A	Efficacious[a] *Nonefficacious*[b]	*Nonefficacious (F, D)*	Efficacious (F) Insufficient evidence (D)
	Safety	Acceptable risk without specialized monitoring				
	Practice implications	Investigational	N/A	Clinically useful[a] *Not useful*[b]	*Not useful (F, D)*	Clinically useful (F) Investigational (D)
Tolcapone	Efficacy	Insufficient evidence	N/A	Efficacious	Insufficient evidence (F, D)	Efficacious (F) Insufficient evidence (D)
	Safety	Acceptable risk with specialized monitoring				
	Practice implications	Investigational	N/A	Possibly useful	Investigational (F, D)	Possibly useful (F) Investigational (D)

Dopaminergic drug targets in Parkinson's disease



Presynaptic targets include l-DOPA substitution combined w/ peripherally active inhibitors of aromatic amino acid decarboxylase (AADC) or catechol-Omethyltransferase (COMT). Monoamine oxidase type B (MAOB) inhibitors enhance synaptic availability of dopamine (both endogenous & exogenous), whereas dopamine agonists act postsynaptically. Dashed arrow from blood to brain designates blood–brain barrier (BBB) transport of l-DOPA. Dashed arrow through dopamine transporter (DAT) denotes reuptake of dopamine from the synaptic cleft. **KEY:** 3-O-M-DOPA, 3-O-methyl-DOPA; D1R, dopamine D1 receptor; DOPAC, 3,4-dioxy-phenylacetic acid; TH, tyrosine hydroxylase. (Poewe et al, 2018 NRDP)

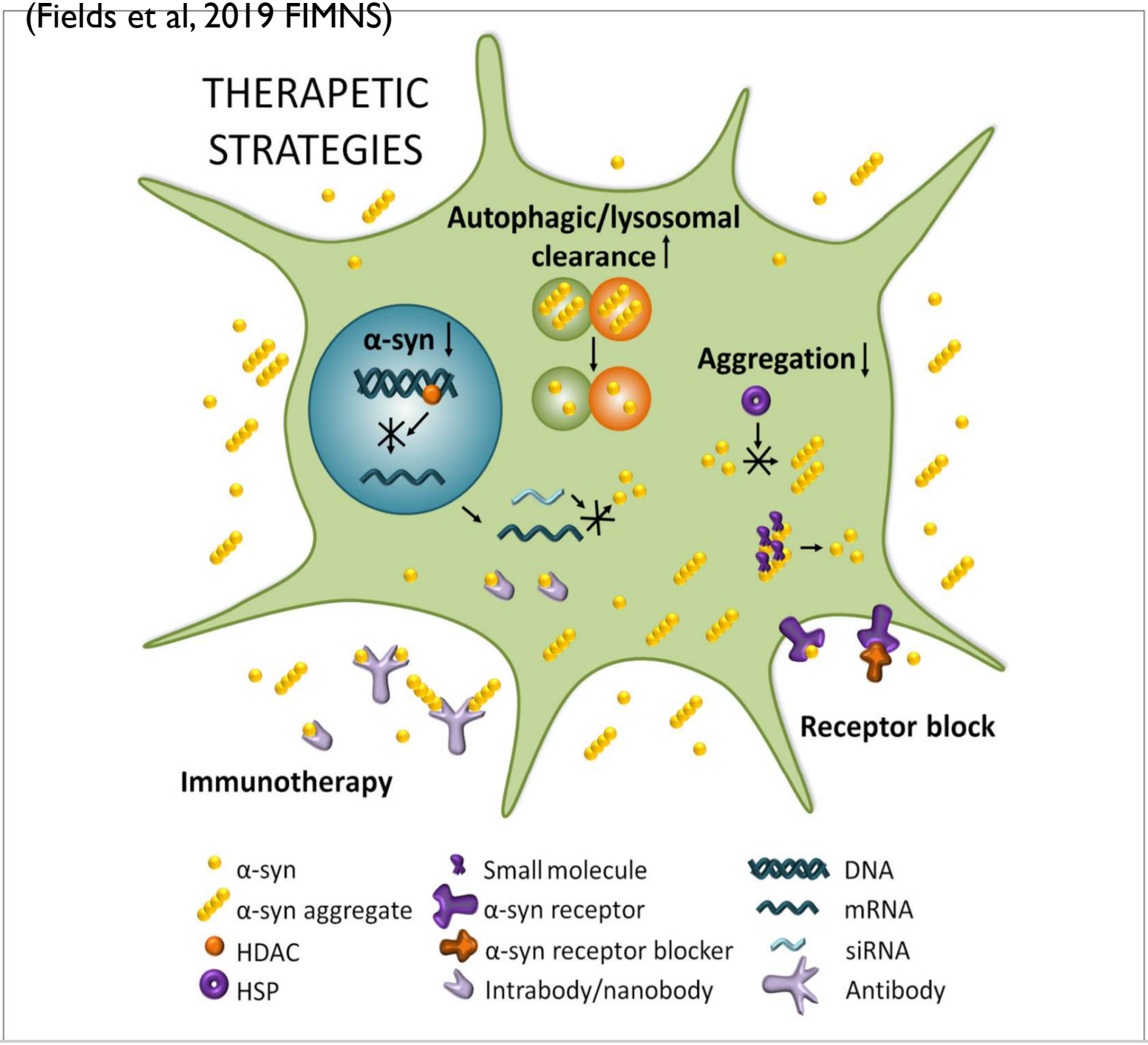
Deep Brain Stimulation for Parkinson Disease – JAMA Expert Consensus Review of Key Issues (2011)

- 1) Patients with PD w/out significant active cognitive or psychiatric problems who have medically intractable motor fluctuations, refractory tremor, or intolerance of medication adverse effects are good candidates.
- 2) Surgical complication rates are *extremely variable*, w/ infection being the most commonly reported complication of DBS. Morbidity ~1-3% Mortality ~0.5%
- 3) Deep brain stimulation programming is best accomplished by a highly trained clinician and can take 3 to 6 months cut-and-paste to obtain optimal results.
- 4) DBS improves L-dopa-responsive symptoms, dyskinesia, & esp. tremor and on-off; benefits long-lasting in classic motor domains. ~50-60% med reduction with 50% improvement in classic UPDRS compared to non-med state.
- 5) Subthalamic nuclei DBS can be complicated by ↑depression/apathy, impulsivity, worsened verbal fluency, & executive dysfunction in a subset of pts.
- 6) Both globus pallidus interna and subthalamic nuclei DBS have been shown to be effective in addressing the motor symptoms of PD.
- 7) Ablative therapy still an effective alternative, considered in a select group of appropriate patients, but generally as ~last resort. MJ Fox had early palladal Abl.

Espay et al, 2019 *Neurology* pose a cogent set of questions for the entire field of NDD, not just PD!

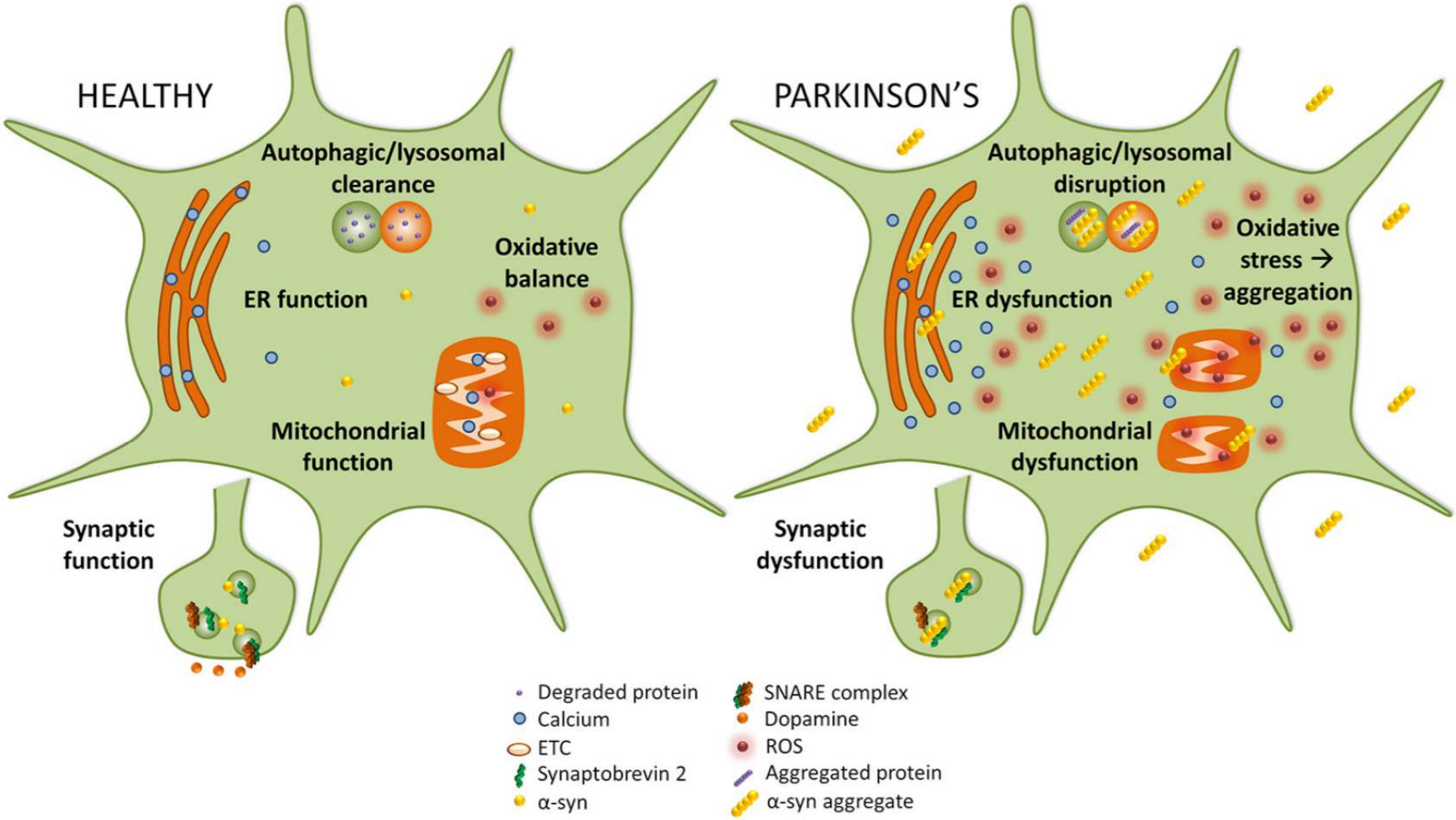
- ***A fundamental question in advancing Parkinson disease research is whether it represents one disorder or many***. Does each genetic PD inform a common pathobiology or represent a unique entity? Do similarities between genetic & idiopathic forms of PD outweigh differences? If aggregates of α-synuclein in Lewy bodies/Lewy neurites are present in most, are they also etio-pathogenically significant in each (α-syn pathogenesis)? What does it mean that postmortem studies in PD demonstrate that mixed protein-aggregate pathology is the rule and pure α-synucleinopathy the exception? Should we pursue convergent biomarkers representative of the diverse whole of PD or subtype-specific divergent biomarkers present in only some not others?
- Have clinical trials failing to demonstrate efficacy of putative disease-modifying interventions been true failures (shortcomings of hypotheses, which should be rejected) or false failures (shortcomings of the trials; hypotheses should be re-tested/preserved)?
- These questions reflect nosologic struggle btwn a lumper's clinicopathologic model embracing heterogeneity of one disease and the splitter's focus on a pathobiology-specific set of diseases.
- Most important, even if PD is not a single disorder, can advances in biomarkers & disease modification be revised to concentrate on pathologic commonalities in large, clinically defined populations? Or should our efforts focus on smaller subgroups of patients, distinguished by well-defined molecular characteristics, regardless of their phenotypic classification?
- Will our clinical trial constructs be revised to target larger and earlier, possibly even prodromal, cohorts? Or should efforts be reconstructed to target smaller but molecularly defined presymptomatic or postsymptomatic cohorts?

(Fields et al, 2019 FIMNS)



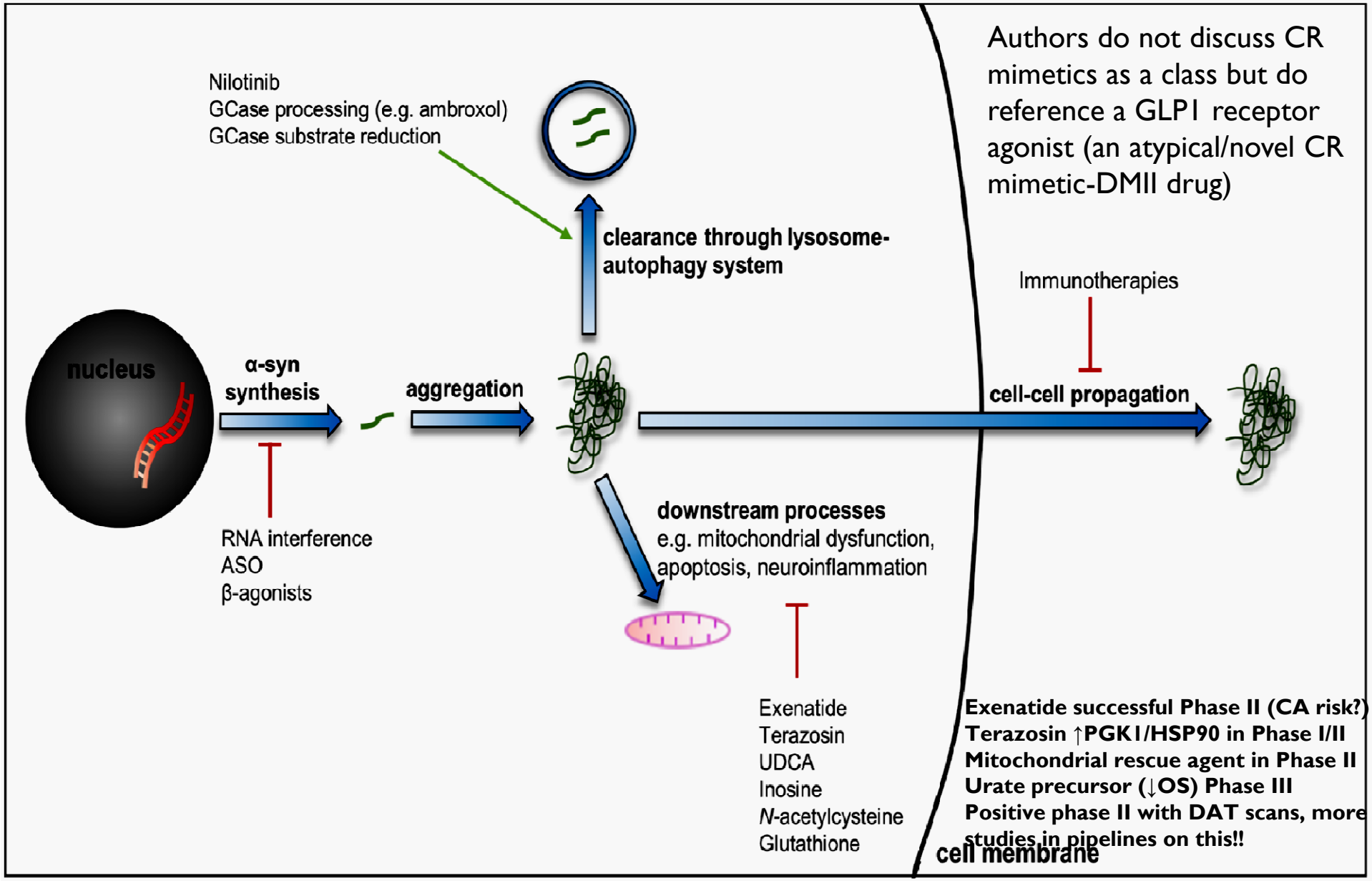
Therapeutic strategies:
1) Boosting autophagic/ lysosomal clearance of α-syn/its various aggregates before they disrupt cellular homeostasis.
2) Reducing SNCA mRNA by modulating histone deacetylase (HDACs) or via RNA interference (RNAi) to decrease α-syn.
3) Reducing aggregation by ↓ multimerization of α-syn i.e., through heat shock proteins (HSPs) or by dissociating existing aggregates with small molecules.
4) Blocking α-syn entry via receptor blocking would target the spread of α-syn, preventing its transport from cell to cell.
5) Immunotherapy might neutralize α-syn and α-syn aggregates extracellularly (and even intracellularly in the case of intra/ nanobodies).
(Fields et al, 2019 FIMNS)

Other cellular/molecular targets in PD



Implicated pathways for α-syn toxicity. (L) healthy cellular pathways are illustrated, on (R) pathways perturbed in PD. Autophagy is blocked, causing accumulation of aggregated protein →↑ aggregation. In PD ER stress leads to Ca++ efflux into cytoplasm. In PD, electron transport chain (ETC) & MITO fxn are compromised, causing increase in oxidative stress. α-syn may interact w/synaptobrevin-2→synaptic dysfxn.

On the 'cutting-edge' . . . or is it the 'crumbling edge' of new treatments (Stoker & Barker, 2020)



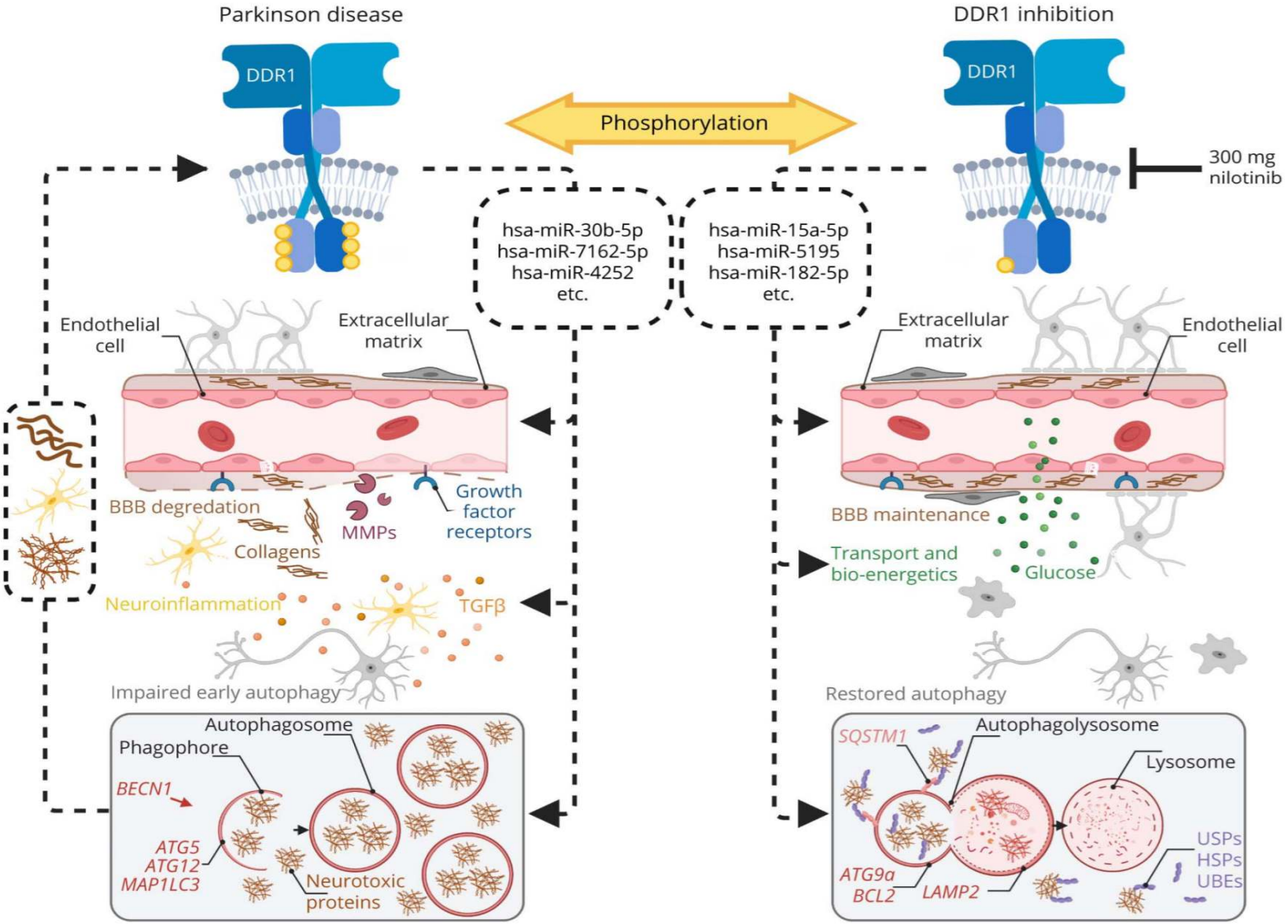
Drug/class	Proposed mechanism	Progress in trials
α-synuclein reduction		
β-agonists	Reduced α-synuclein transcription through acetylation of promoters and enhancers of the *SNCA* gene[28]	Not started
Nilotinib	Inhibition of ABL tyrosine kinase activity and enhanced autophagy[34]	Safe and tolerable but no clinical benefit in phase II trial
Terazosin	Activation of PGK1 and HSP90, increased ATP levels, and reduced α-synuclein levels[35]	Single-centre randomised placebo-controlled trial currently enrolling patients
Mitochondrial function		
Ursodeoxycholic acid	Restoration of mitochondrial function	Randomised placebo-controlled trial currently recruiting patients
N-acetylcysteine	Antioxidant effect and elevation of glutathione levels[36]	Small open-label phase II study showed no changes in indicators of oxidative damage or brain glutathione levels[36]
Glutathione	Reduction in reactive oxygen species and free radical levels	Double-blind trial completed, with no clinical benefit demonstrated over placebo
Neuroinflammation		
Azathioprine	Modulation of peripheral immune system profile	Single-centre randomised placebo-controlled trial about to start enrolling patients
Sargramostim (G-CSF)	Induction of Treg immune responses[37]	Phase I placebo-controlled trial completed Generally well tolerated, with reported modest improvement in UPDRS part III scores[38]
AZD3241	Reduced oxidative stress and neuroinflammation through inhibition of myeloperoxidase	Phase 2a randomised placebo-controlled trial completed Safe and well tolerated with reduced nigrostriatal distribution of microglia[39]
Other		
Inosine	Elevation of urate levels	Randomised placebo-controlled phase III trial halted early in 2018, with results awaited
Exenatide	GLP-1 receptor activation leading to inhibition of apoptosis, reduced microglial activation and neuroinflammation, reduced oxidative stress, and promotion of neurogenesis	Well tolerated, with improvements seen in UPDRS part III scores in randomised controlled trial[40] Phase III trial currently in set-up
Isradipine	Neuroprotection through blockade of L-type calcium channels in substantia nigra[41]	Multicentre phase III trial recently completed, with no improvement in motor or quality of life outcomes
Deferiprone	Iron chelation	Phase II randomised double-blind placebo-controlled trial completed, demonstrating reduced iron content in caudate and dentate nucleus No significant clinical benefit[42]

Status of putative disease modifying agents (Stoker & Barker, 2020)

Latest bad news (Feb, 2021CTA) re: antibodies as therapies in neurodegenerative disorders

- This month has seen failure of main assets in the Parkinson's disease (PD) pipeline. On 3 February, Biogen announced the discontinuation of its monoclonal antibody (mAb) pipeline drug cinpanemab following its failure to achieve primary and secondary endpoints in the Phase II proof-of-concept trial called SPARK in Parkinson's disease (PD), investigating cinpanemab in 357 PD patients. Endpoint: drug impact on Unified Parkinson's Disease Rating Scale (MDS-UPDRS) after 52 and 72 weeks vs. placebo.
- Sanofi announced on Feb 5 that its pipeline asset, venglustat (a glucosylceramide synthase inhibitor targeting effects of a GBA mutation found in up to 10% of PD patients, associated w/earlier disease) failed in its Phase II trial, leading the company to stop further development. The trial enrolled 270 patients, assessing MDS-UPDRS over 52 weeks.
- The PD pipeline was hit with a string of failures among its most anticipated mid-stage disease-modifying therapies (DMTs). This news presents a challenge for similar pipeline candidates undergoing development. It also amplifies a major unmet need in bringing DMTs & neuroprotective agents to this market, which is currently dominated by symptomatic treatments.
- With Roche's Prasinezumab being the only mAb advancing into late-stage Phase IIb, results of its upcoming late-stage trial are now even more critical to the development of novel mechanisms of action (MOA) and strategies in the future treatment of PD. Roche is currently developing its mAb prasinezumab after it has provided just enough evidence to proceed into a Phase IIb trial. It has a novel MOA similar to Biogen's cinpanemab: both target the α-synuclein protein, a hallmark pathological factor in PD, preventing their aggregation and potentially halting disease progression. This MOA was unanimously highly regarded by key opinion leaders (KOLs) interviewed by GlobalData, stating that this approach, if approved, has potential to revolutionise the treatment of PD.
- However, some were concerned that it might still be unclear whether targeting extracellular α-synuclein protein would offer enough benefit to PD patients. As such, all eyes will be on Roche's prasinezumab trial as the remaining hope to resurrect the α-synuclein hypothesis.
- Does any of this sound familiar?

A recent Phase II study (Fowler et al. 2021 in *Neurology*) of Nilotinib, 300 mg DDR1 inhibitor (R for tyrosine kinase) over 1 yr: rescued BBB, ↓ inflammation, ↑ autophagy, many miRNA Δ. Neuroprotective w/preserved cognition.



VV-mediated expression of various growth factors or neurotransmitter-synthesizing enzymes. Stem/fetal cell Rx

- Glial derived neurotrophic factor (GDNF) to protect nigral DA neurons & regenerate axons - a small open-label trial w/injections into putamen generated buzz but larger placebo-controlled trial w/smaller doses failed to show benefit. Neurturin, (member of GDNF family) also failed in RCT, but post-mortem showed that neurturin only expressed in relatively few cells surrounding injection site. AD parallels here too w/NGF?
- Bartus and Johnson 2016: "(1) unexpected and undesirable side effects, at times serious, have plagued many efforts to deliver neurotrophic factors to humans; (2) the magnitude & consistency of clinical benefit has been disappointing; (3) by far, most consistently proposed reason for the side effects & poor efficacy has been inadequate dosing and delivery."
- How are side effects explained by too low a dose? What does failure here mean? *Target earlier stage PD, when more nigrostriatal axons remain functional or does pro-geroid environs of PD overwhelm/cancel out GF effects?*
- VV-mediated expression of enzymes in DA pathways. Vectors →↑ tyrosine hydroxylase with cofactors & AADC injected into striatum. Genetically modifies cells in striatum so that they can produce & release dopamine locally, either from tyrosine or from administered l-DOPA. Animal studies demonstrated that approach provides relief of DA-dependent motor symptoms, but w/constant dopamine receptor stimulation might also reduce the risk of motor fluctuations developing later on.
- Another approach has targeted subthalamic nucleus with AAV2 vector-mediated delivery of glutamate decarboxylase to induce GABAergic inhibition of subthalamic nucleus, with promising results in sham surgery Phase II.
- Other more exotic approaches such as fetal cell transplants and stem cell transplants have seen waning enthusiasm – FC DA transplants → induction of refractory dyskinesias

Gene therapies in Parkinson's disease –

DA synthesis regulated by 3 rate-limiting enzymes: GTP cyclohydrolase 1 (GCH1), tyrosine hydroxylase (TH), aromatic amino acid dopa decarboxylase (AADC), with AADC as final enzymatic step. Clinical trials with AAV2-AADC have demonstrated modest improvement in symptoms. A more targeted delivery of AAV2-AADC by real-time MRI-guidance was also safe/well tolerated. A lentivirus-based gene therapy delivering all 3 enzymes (TH, AADC, & GCH1) was well-tolerated in Phase 1/2 trials, with moderate improvements n sxns.

GABA	AAV2-*GAD*	IP (subthalamic nucleus)	NCT00195143	1	Idiopathic PD, > 5 years of disease duration	Safety	2003-2005	90
	AAV2-*GAD*	IP (subthalamic nucleus)	NCT00643890	2	Idiopathic PD, > 5 years of disease duration	Disease severity and progression	2008-2010	91,151
Dopamine	Lentivirus-*TH/AADC/CH1*	IP (striatum)	NCT00627588	1/2	Bilateral idiopathic PD, age 48-65, > 5 years of disease duration	Safety	2008-2012	86
	Lentivirus-*TH/AADC/CH1*	IP (striatum)	NCT01856439	1/2	From NCT00627588	Long-term safety and tolerability	2011-2022	86
	AAV2-*AADC*	IP (striatum)	NCT00229736	1	Mid- to late-stage PD, ≤ 75 years of age	Safety and tolerability	2004-2013	83
	AAV2-*AADC*	IP (striatum)	NCT01973543	1	Idiopathic PD, > 5 years of disease duration	Safety and tolerability	2013-2020	152
	AAV2-*AADC*	IP (putamen)	NCT02418598	1/2	Idiopathic PD, < 75 years of age	Safety	2015-2018	
	AAV2-*AADC*	IP (striatum)	NCT03065192	1	Idiopathic PD, > 5 years of disease duration	Safety and suicide risk	2017-2021	
	AAV2-*AADC*	IP (striatum)	NCT03562494	2	PD, age 40-75, > 4 years of disease duration	Change of time in troublesome dyskinesia	2018-2020	153

Promising PD animal model results with cheap and common spice – Cinnamon

- **Abstract - Reduction of Lewy Body Pathology by Oral Cinnamon**
- J Neuroimmune Pharmacol 2021 Sep;16(3):592-608
- Sumita Raha, Debashis Dutta, Avik Roy, Kalipada Pahan α-Synucleinopathies comprise of several neurodegenerative disorders that include Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), characterized by accumulation of aggregated insoluble α-synuclein (α-syn) protein. To date no effective Rx is available to reduce the burden of LB pathology.
- The present investigation underlines the importance of a naturally used spice and flavoring agent viz. cinnamon in reducing α-syn deposits in transgenic mice expressing mutant A53T human α-syn.
- Upon oral administration, cinnamon markedly reduced the level of insoluble α-syn in SN, HC, and brain stem of A53T mice. We also demonstrated that sodium benzoate (NaB), a metabolite of cinnamon, a widely used food additive and a FDA-approved drug (glycine encephalopathy), was also capable of reducing α-syn deposits in A53T mice. In addition, both cinnamon and NaB treatments showed improvement in their motor and cognitive functions.
- Glial activation plays an important role in pathogenesis of various NDD, including PD, DLB and MSA, and we found suppression of microglial/astroglial activation in SN of A53T mice w/cinnamon Rx.
- Neuroprotective proteins like DJ-1 & Parkin known to reduce formation of LB in CNS. Accordingly, we observed upregulation and/or normalization of DJ-1 and Parkin in SN of A53T mice by treatment w/cinnamon & NaB. Together, results highlight a new therapeutic property of cinnamon, that cinnamon/NaB may be used to halt the α-synucleinopathies.

Intranasal delivery of mitochondria for treatment of Parkinson's Disease model rats lesioned with 6-hydroxydopamine

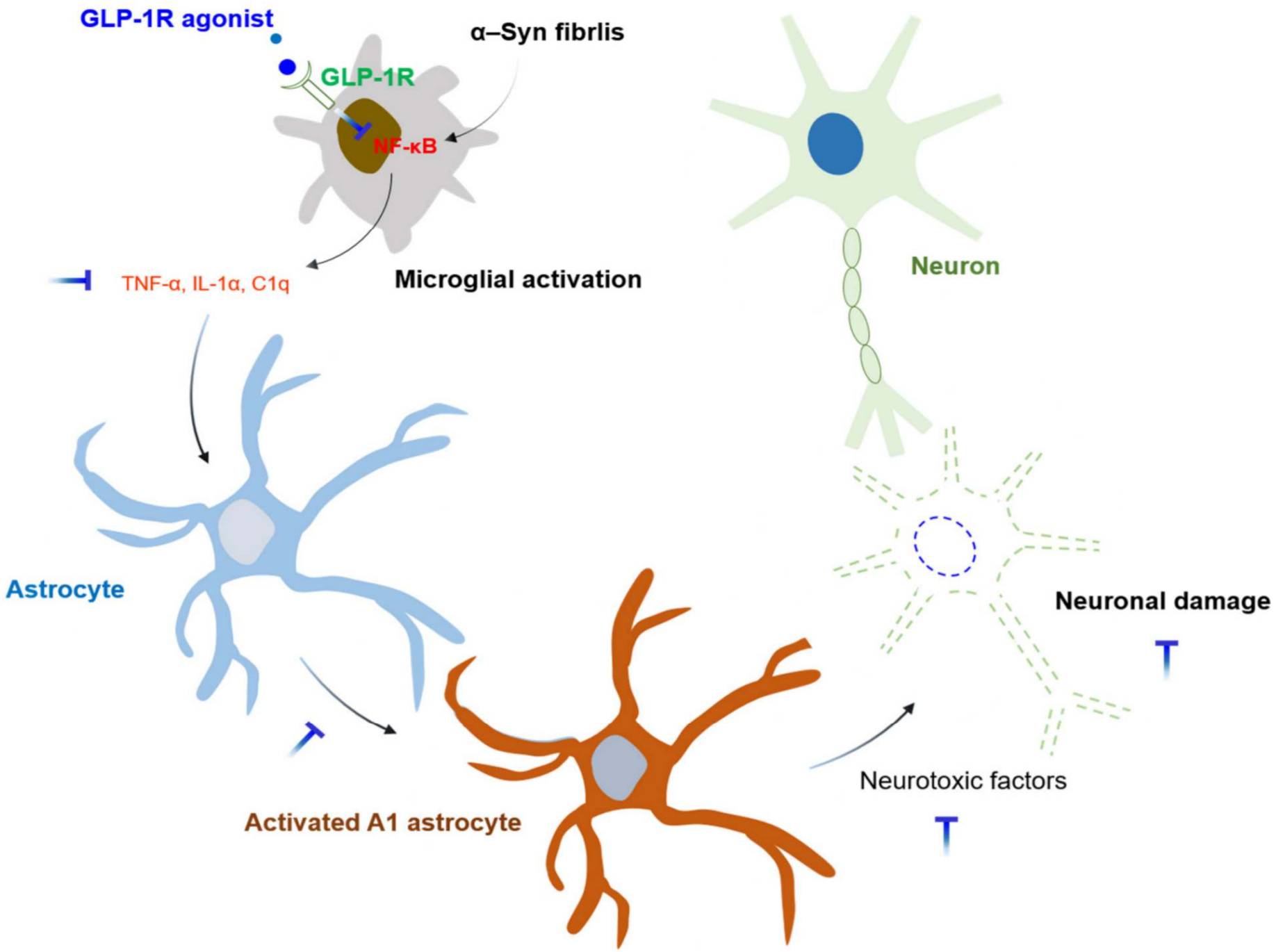
- Jui-Chih Chang, Yi-Chun Chao, Huei-Shin Chang, Yu-Ling Wu, Hui-Ju Chang, Yong-Shiou Lin, Wen-Ling Cheng, Ta-Tsung Lin & Chin-San Liu ***Scientific Reports*** vol. 11, (2021)
- **Abstract**
- The feasibility of delivering mitochondria intranasally to bypass blood–brain barrier in treating Parkinson's disease was evaluated in unilaterally 6-OHDA-lesioned rats. Intranasal infusion of allogeneic mitochondria conjugated w/ Pep-1 (P-Mito) or unconjugated (Mito) performed 1/wk on ipsilateral sides of lesioned brains for 3 mo.
- A significant improvement of rotational and locomotor behaviors in PD rats was observed in both mitochondrial groups, compared to sham or Pep-1-only groups.
- Dopaminergic (DA) neuron survival & recovery > 60% occurred in lesions of SN and striatum in Mito and P-Mito rats. The treatment effect was stronger in the P-Mito group than the Mito group, but the difference was insignificant.
- This recovery associated w/restoration of mitochondrial function & attenuation of oxidative damage in lesioned SN. Notably, P-Mito suppressed plasma levels of inflammatory cytokines. Mitochondria penetrated the accessory olfactory bulb and doublecortin-positive neurons of the rostral migratory stream on ipsilateral sides of lesions and were expressed in striatal, but not SN DA neurons, of both cerebral hemispheres, evidently via commissural fibers.
- This study shows promise for intranasal delivery of mitochondria, confirming mitochondrial internalization & migration via RMS neurons in OB for PD therapy.

CR, Exercise, and CR Mimetics in PD – still a largely unexplored space in neuroprotection?

- Exercise effective in MPTP AM of PD in reducing risk, sxns & progression, clinical studies suggest protective benefits, compliance and blinding are issues.
- GLP-1 agonists effective in AM.

Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease

- Seung Pil Yun, Tae-In Kam, Han Seok Ko *Nature Medicine* volume 24, 931–938 (2018)
- Activation of microglia by inflammatory mediators can convert astrocytes into a neurotoxic A1 phenotype in a variety of neurological diseases. Development of agents inhibiting formation of A1 reactive astrocytes could be used to treat these diseases for which there are no disease-modifying therapies. Glucagon-like peptide-1 receptor (GLP1R) agonists have been indicated as potential neuroprotective agents for neurologic disorders such as Alzheimer's disease and Parkinson's disease.
- Mechanisms by which **GLP1R agonists** are neuroprotective are **not known**. (!)
- Here we show that a potent, brain-penetrant long-acting GLP1R agonist, NLY01, protects against the loss of dopaminergic neurons and behavioral deficits in the α-synuclein preformed fibril (α-syn PFF) mouse model of sporadic Parkinson's disease
- NLY01 also prolongs the life and reduces behavioral deficits & neuropathological abnormalities in the human A53T α-synuclein (hA53T) transgenic mouse model of α-synucleinopathy-induced neurodegeneration.
- We found that NLY01 is a potent GLP1R agonist with favorable properties that is neuroprotective through the direct prevention of microglial-mediated conversion of astrocytes to an A1 neurotoxic phenotype.
- In light of its favorable properties, NLY01 should be evaluated in the treatment of Parkinson's disease and related neurologic disorders characterized by microglial activation.


GLP-1R agonist
α–Syn fibrlis
GLP-1R
NF-κB
TNF-α, IL-1α, C1q
Microglial activation
Neuron
Astrocyte
Neuronal damage
Activated A1 astrocyte
Neurotoxic factors

Neurodegeneration sits at a complex intersection of genetically & environmentally exacerbated aging challenges/phenotypes

- Despite relative prohibition against apoptosis in neurons, neurons in NDD undergo progressive synaptic & cell loss. ***How to explain?***
- Aging degrades proteostasis/autophagy/MITO (classic aging phenotypes), while disinhibiting INFLAM ('inflammaging'). These constitutive of NDD.
- Aging may recruit ↑expression of several proteins (amyloid, tau, alpha synuclein, etc.) in effort to compensate for age-related network declines.
- Most proteins implicated in NDD constitute intrinsically difficult challenges for autophagy systems, suggesting ***slim margins for safety***, where various factors (genetic & environmental) create initial cascade of autophagy overload and then ATG failure w/resulting proteinopathy.
- As autophagy fails, both MITO dysfunction and disinhibited (not kind to CNS) INFLAM (also both aging phenotypes) are then also exacerbated.
- Mutually reinforcing effects of proteinopathy, mitochondrial dysfunction, inflammation, and eventually a spreading cellular senescence → first local synaptic/neural loss & over time → increasingly global network failure.
- ***Do commonalities rather than differences between NND*** best explain neurodegeneration - early Rx rescuing ATG, MITO fxn, ↓INFLAM?