Getting involved in Parkinson's disease

DEVELOPING AND EXPLOITING *C. ELEGANS* MODEL FOR PARKINSON'S DISEASE
Supported by the PD society

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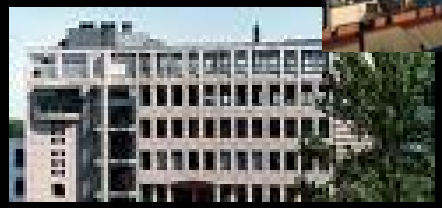
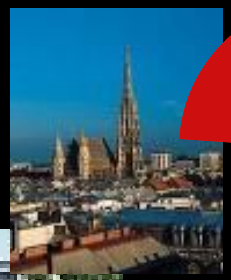
A. GARTNER lab's website http://www.dundee.ac.uk/biocentre/SLSBDIV3ag.htm

The long way to Dundee

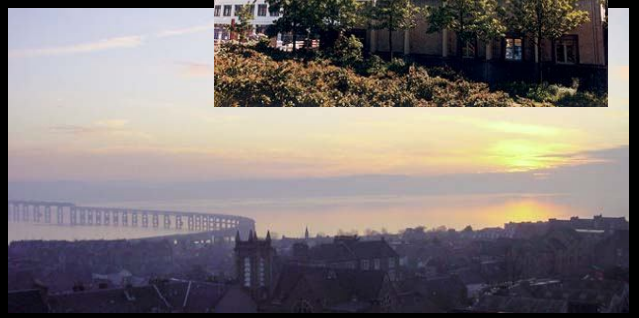


Imp, Vienna, Austria



Cold Spring Harbor, New York

MPI, Munich, Germany



University of Dundee

Talk

- **Brief definition of Parkinson's Disease (PD)**

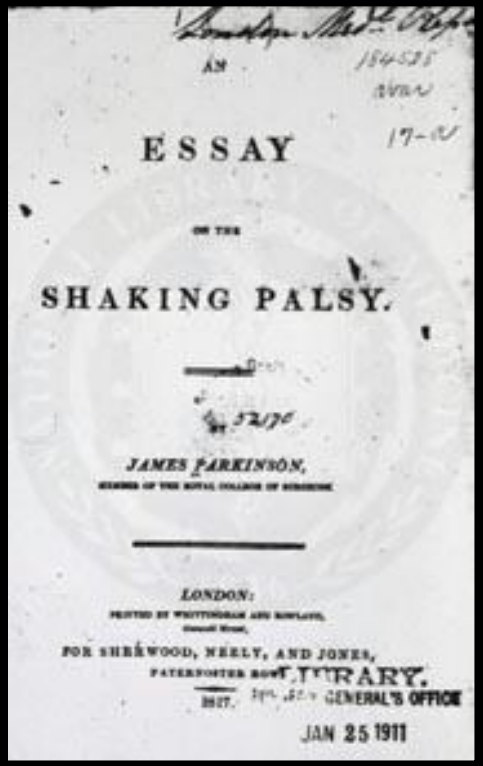
- **Human Parkinson's Disease genetics**
  Human genetics

- ***Exploring C. elegans* as a model for Parkinson's Disease**

Why is it so difficult to give this talk

- I have family members affected by neurodegenerative diseases
- Hope for a cure, follow up on internet and literature
- Why is it so difficult to find a cure against neurodegenerative disease as opposed to “say infectious diseases” eg. “antibiotics”
- Why is basic science so important and relevant to disease models even if these models do not appear to be directly connected to affected patients at the first glance.
- Please bare in mind that I am not a medical doctor

Parkinson's Disease (PD): motor symptoms


AN

ESSAY

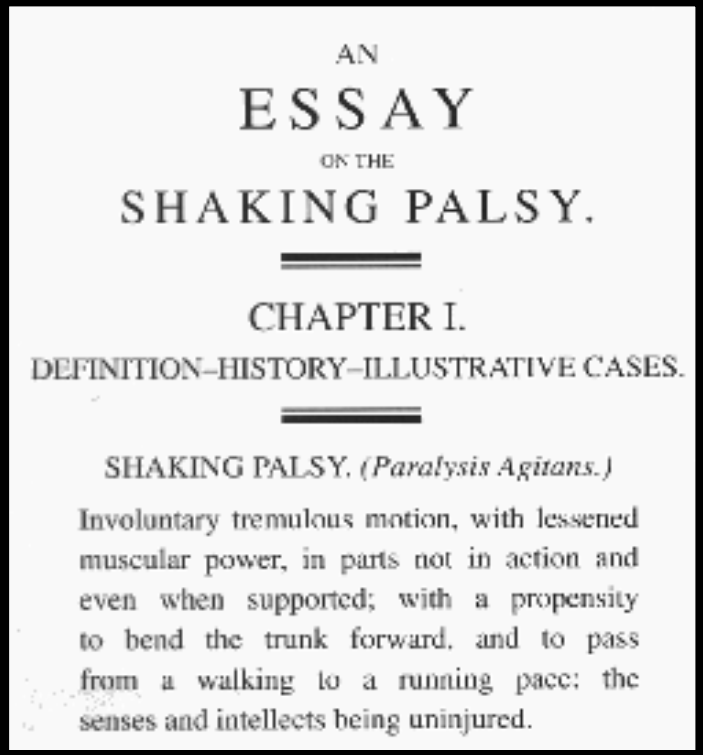
ON THE

SHAKING PALSY.

JAMES PARKINSON,

LONDON:

FOR SHERWOOD, NEELY, AND JONES,


AN

ESSAY

ON THE

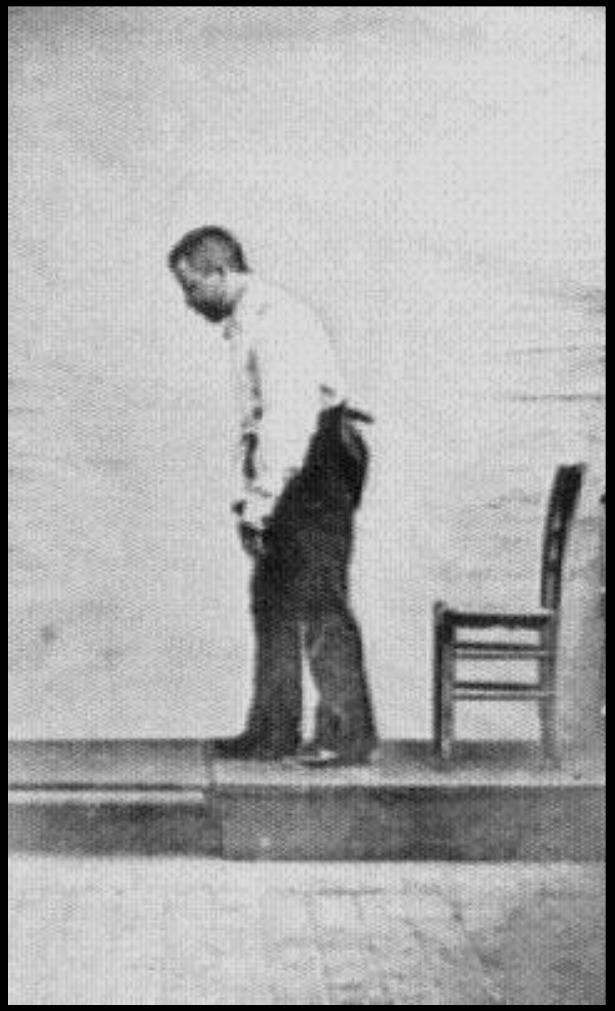
SHAKING PALSY.

CHAPTER I.

DEFINITION–HISTORY–ILLUSTRATIVE CASES.

SHAKING PALSY. *(Paralysis Agitans.)*

Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace: the senses and intellects being uninjured.

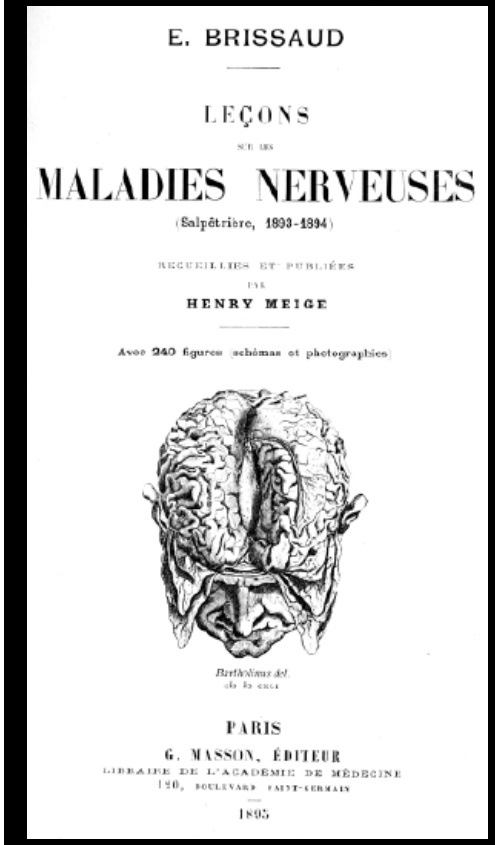


- **Clinical signs:** **-resting tremor**
  -bradykinesia
  -rigidity

Parkinson J. (1817) *An essay on the shaking palsy, Sherwood, Neely and Jones, London*

- Frequent neurodegenerative disease :
  2% population > 65 years
- No curative or preventive treatment
- Effective symptomatic treatment (L-Dopa)
- Largely unknown ethiology

Neuronal loss in the *Substantia Nigra*


E. BRISSAUD

LEÇONS

SUR LES

MALADIES NERVEUSES

(Salpêtrière, 1893-1894)

RECUEILLIES ET PUBLIÉES

PAR

HENRY MEIGE

Avec 240 figures (schémas et photographies)

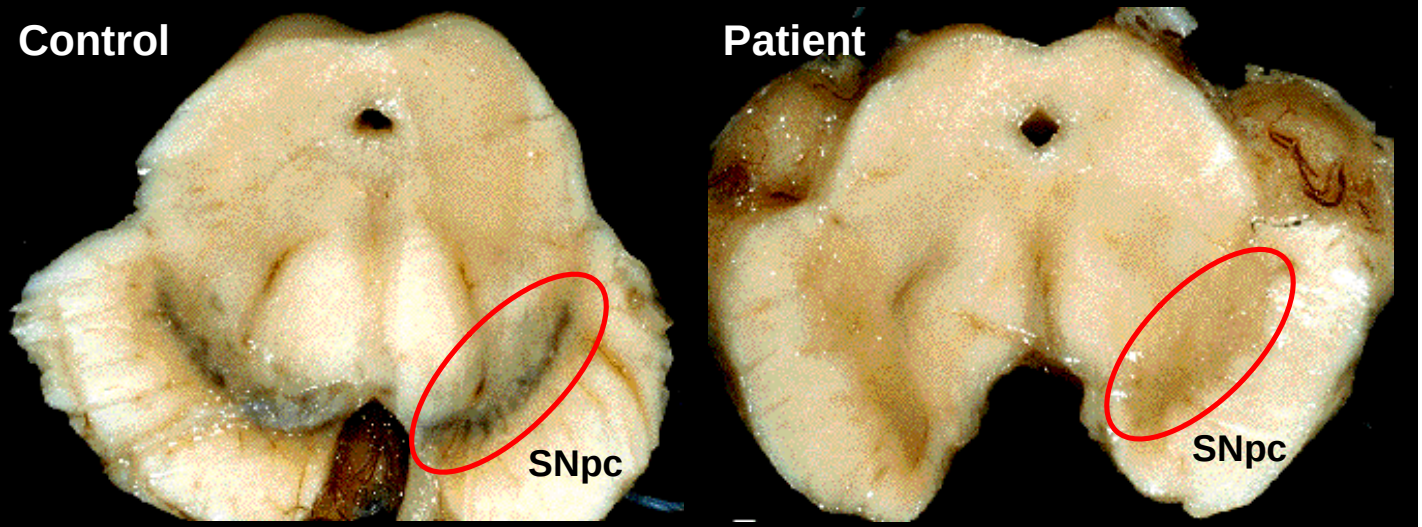
PARIS

G. MASSON, ÉDITEUR

LIBRAIRE DE L'ACADÉMIE DE MÉDECINE

120, BOULEVARD SAINT-GERMAIN

1895



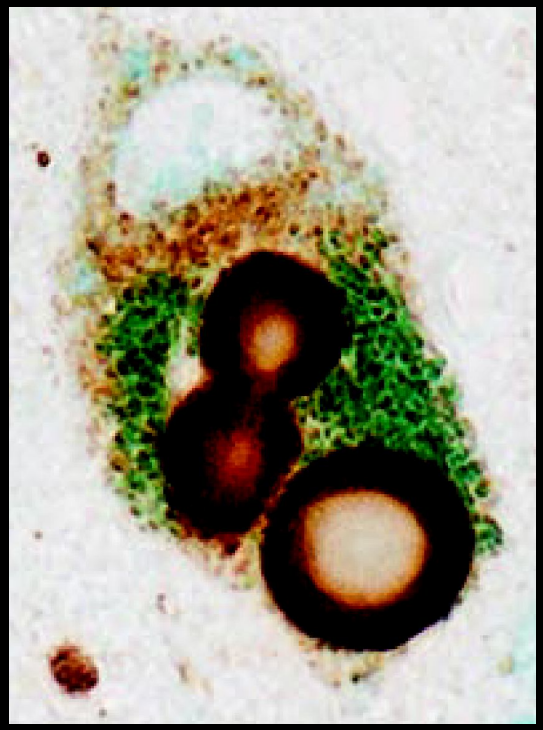
- **Neuropathology:**

death of the neuromelanin containing neurons of the *Substantia Nigra*

Brissaud E. (1895) *Leçons sur les Malades Nerveuses*

Tretiakoff C. (1919) *Thesis*

Intracytoplasmic inclusions: Lewy Bodies



- **Lewy Bodies are aggregates of:**

  organelles

  proteins

- **Not present in all parkinsonian patients**

- **Found in other neurodegenerative diseases:**

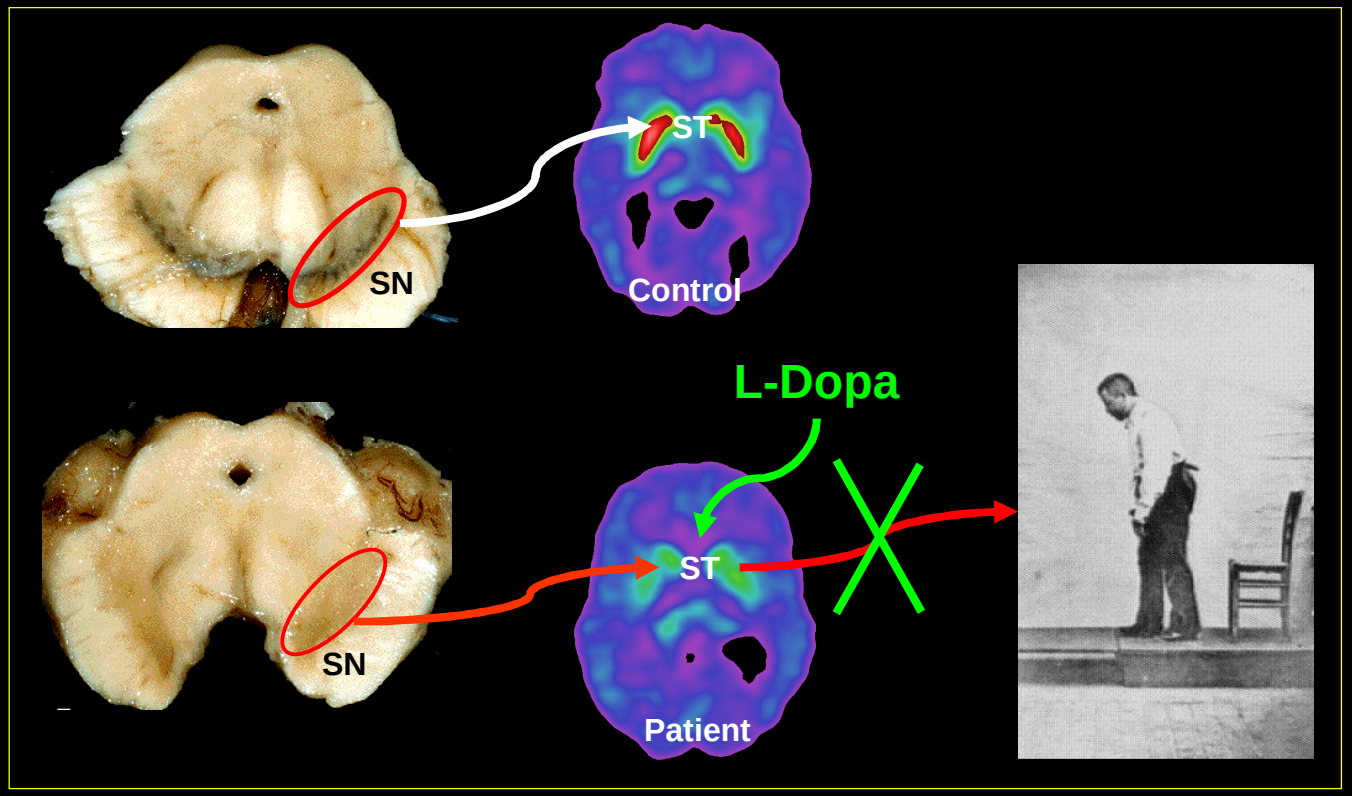
  Synucleinopathies (DLB, LBAD; MSA)

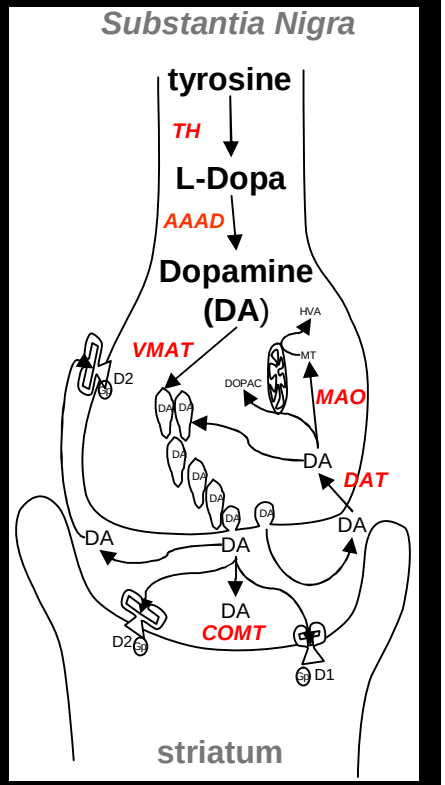
Lewy F.H. (1914) *Dtsch. Z. Nervenheilk.*

Dopaminergic death / L-dopa treatment

- **Dopamine deficit in the striatum caused by the progressive and selective death of the dopaminergic neurons of the *Substantia Nigra***

- **Symptomatic treatment : L-Dopa (levodopa)**





Carlsson A (1959) *Pharmacol. Rev.*

Ehringer H & Hornykiewicz O (1960) *Systems. Klin. Wochenschr.*
Birkmayer W & Hornykiewicz O (1961) *Wien Klin. Wochenschr.*

Fundamental Problem:

How to prevent death of dopaminergic neurons?

we ideally want to take a system apart, see what is wrong fix it and reassemble again, eg broken car

Why is this so difficult for PD compared to infectious disease:

- **symptoms appear rather late**
- **hard to determine what is cause and consequences**
- **hard to identify molecules involved in PD Disease**
- **hard to look into the brain of patients**
- **hard to find model systems**

How can we start to address what is wrong in PD disease?
(taking advantage of rare disease variants)

INTOXICATION

MPTP, pesticides, 6-OHDA

FAMILIAL PD

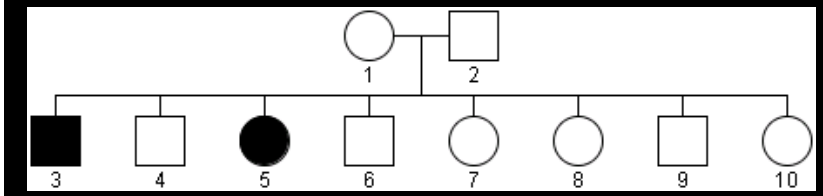
PD patients entering the clinic

FAMILIAL
(rare)

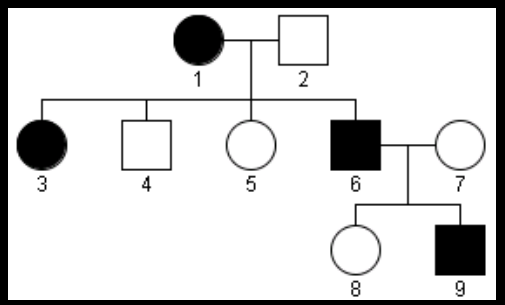
SPORADIC
(no family history)
(most common)

AUTOSOMAL RECESSIVE (AR)

Early Onset



AUTOSOMAL DOMINANT (AD)



“NOT GENETIC”

Mutations in 6 genes cause Parkinson's Disease

Locus	Inheritance	Clinic	Onset age	Gene	Reference
PARK1	Dominant	± dementia	40s	α-SYNUCLEIN	*Polymeropoulos 1997*
PARK4	Dominant	a/typical	40/60	α-SYNUCLEIN	*Singleton 2003*
PARK5	Dominant	typical	50s	UCH-L1	*Leroy 1998*
PARK6	Recessive	Slow progression	30-40	PINK1	*Valente 2004*
PARK7	Recessive	Slow progression	30-40	DJ-1	*Bonifati 2003*
PARK8	Dominant	± dementia	60s	LRRK2	*Paisan-Ruiz 2004*
PARK9	Recessive	Kufor-Rakeb	12-14	ATP13A2	*Ramirez 2006*

How can we start to address what is wrong in PD disease?
(taking advantage of rare disease variants)

INTOXICATION

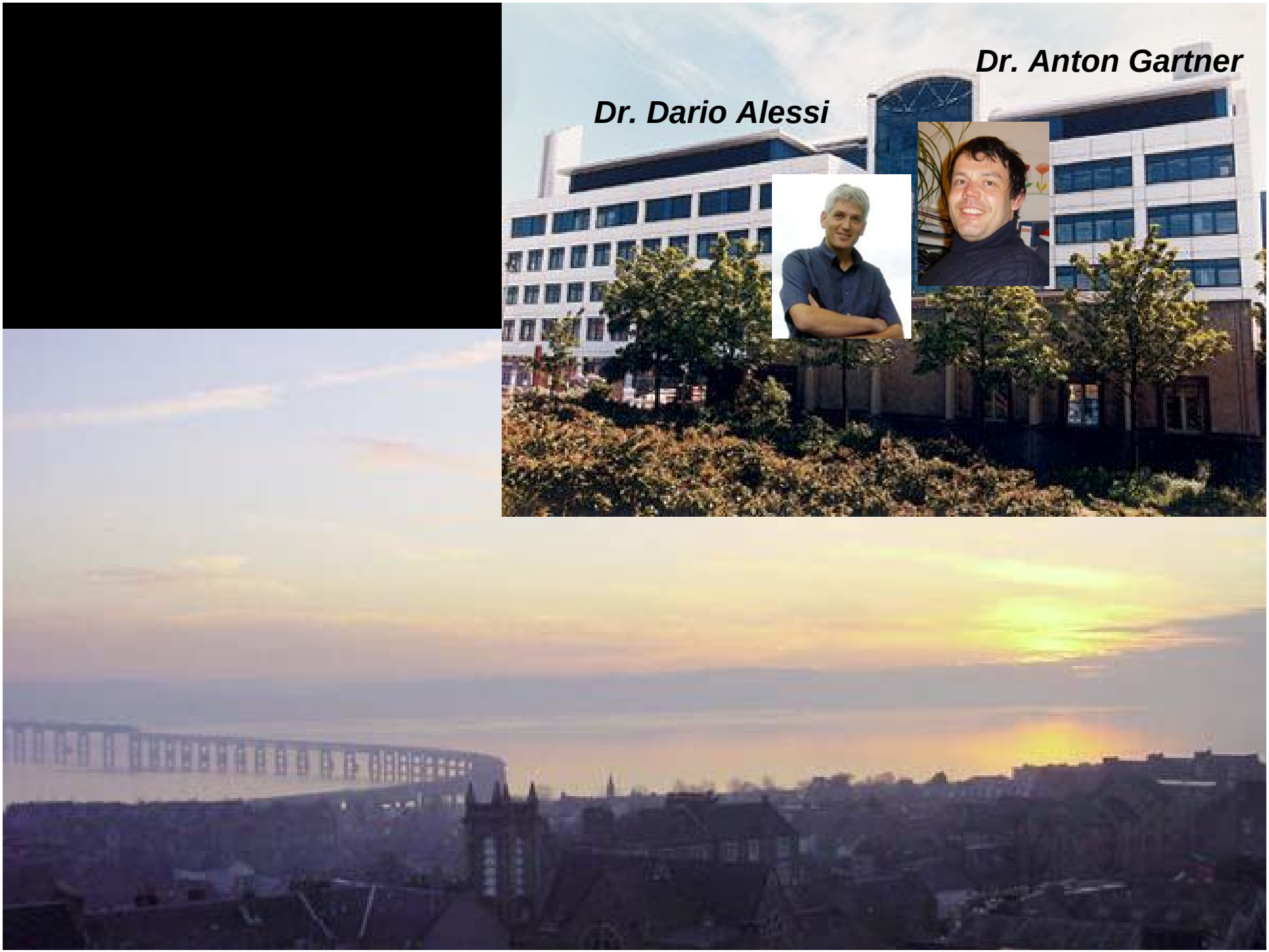
MPTP, pesticides, 6-OHDA

FAMILIAL PD

- **The Case of the Frozen Addicts: How the solution of an extraordinary medical mystery spawned a revolution in the understanding and treatment of Parkinson's disease**
- By J. William Langston and Jon Palfreman. 309 pp. New York, Pantheon, 1996.
-
- This book dramatically recounts the discovery of the cause of a local outbreak of sudden, severe parkinsonism in a group of young adults in northern California and how this discovery led to greater insight into Parkinson's disease. Langston is the Bay Area neurologist who reported the event and led a team of investigators to pinpoint the toxicant responsible for the acute loss of dopamine-containing neurons in the substantia nigra, causing the parkinsonism in these patients. The story unfolds and builds suspense as Langston and his colleagues determine that the toxicant is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP was the unwanted product resulting from

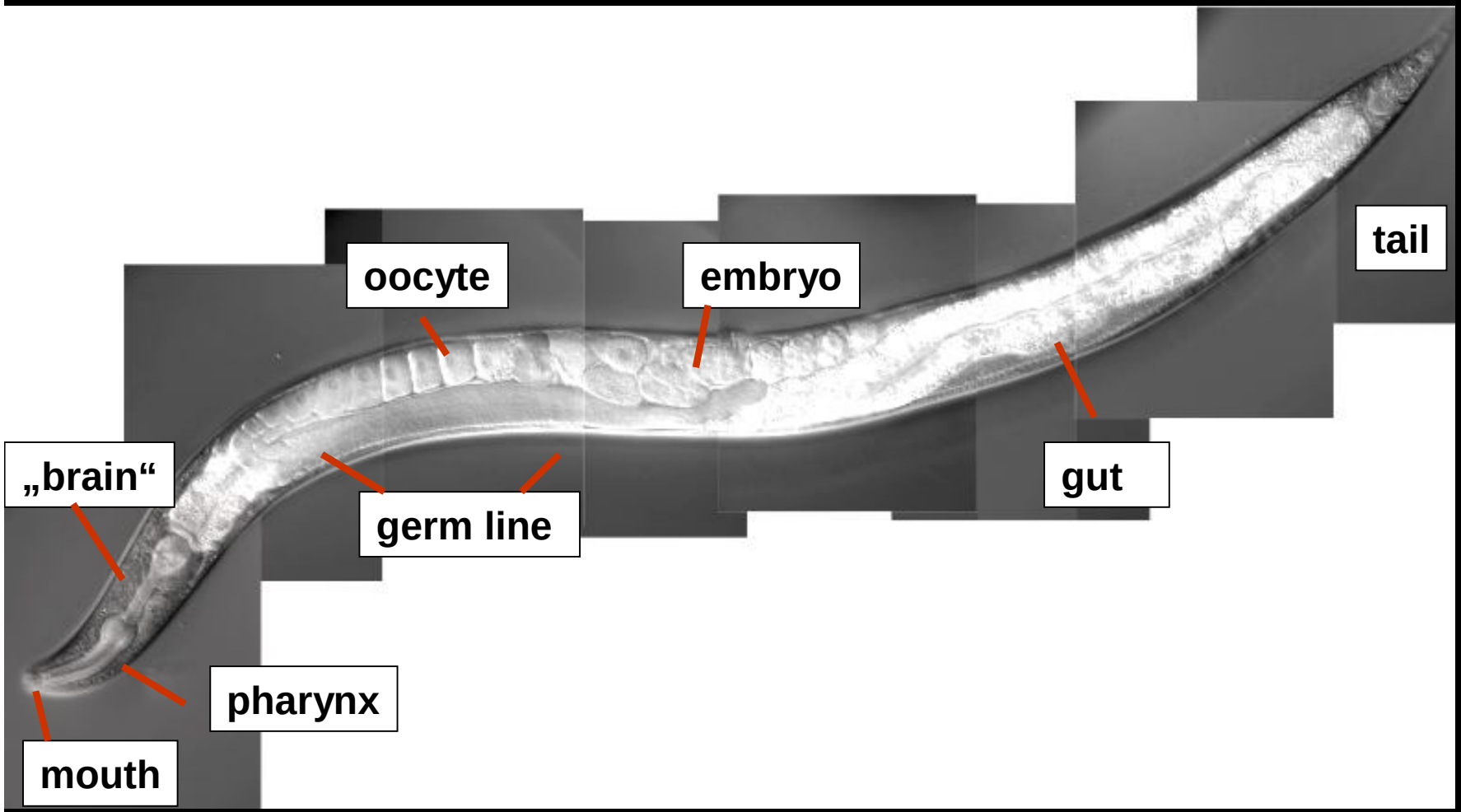
INTOXICATION

Toxins like MPTP and 6-OHDA are directly imported into dopaminergic neurons and cause their degeneration


Dr. Anton Gartner
Dr. Dario Alessi

Why do we use model organisms?

C. elegans worms as a model for Parkinsons disease?


oocyte
embryo
tail
„brain“
germ line
gut
pharynx
mouth
1 mm

2002

Nobel Prices for medicine on “worm” research?

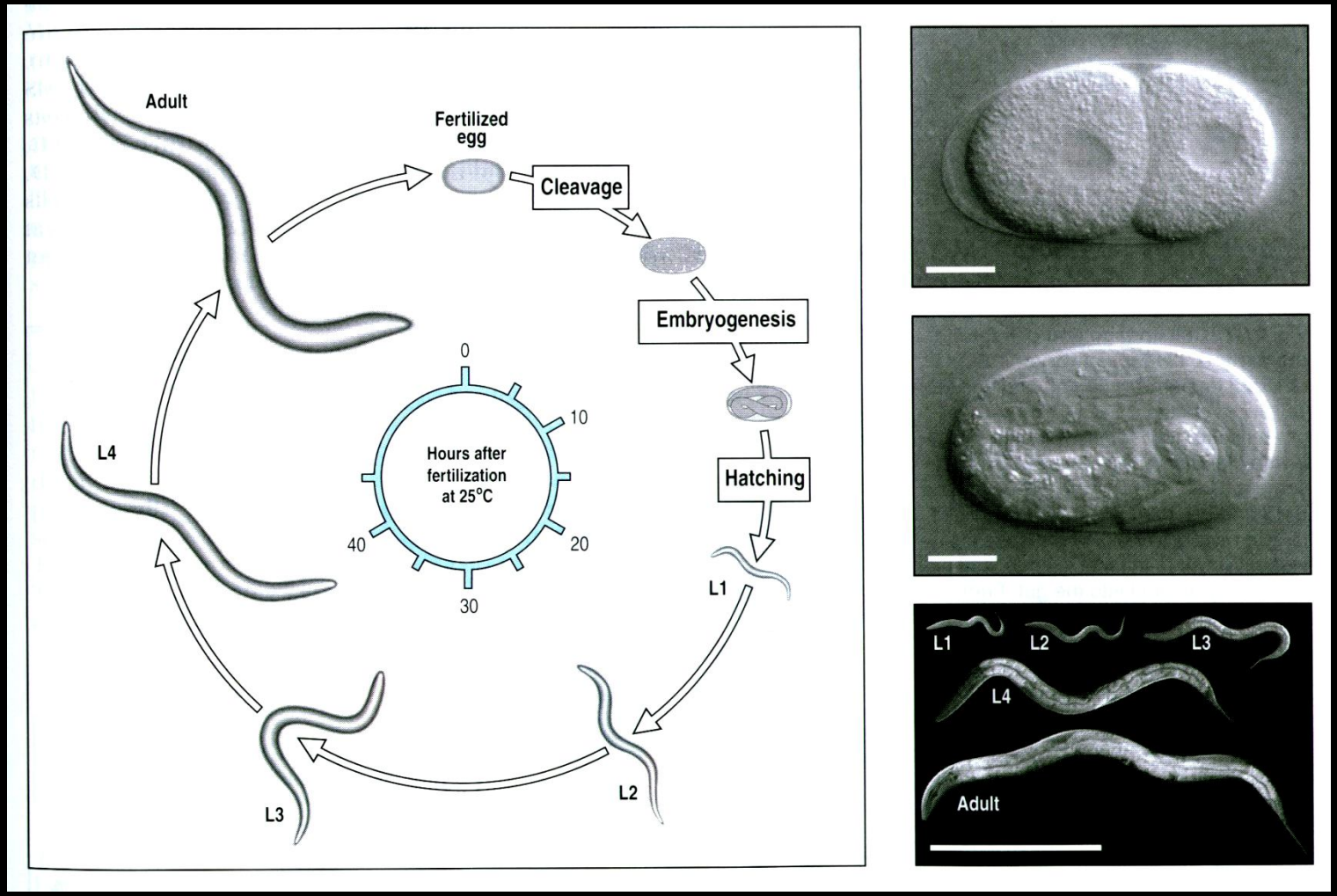
Sydney Brenner
John Sulston
Bob Horvitz

2006

Andy Fire

Craig Mello

Life cycle of *Caenorhabditis elegans*



The newly hatched larva contains all somatic cells of the adult
The gonads develop during the four larval stages L1 to L4

Why worms as a model system ?

- Simple
- Cheap to maintain and easy to propagate
- Amenable to genetic analysis (hermaphrodites/males)
- Short generation time
- Can be frozen away
- Simple, invariant development
- Complete wire diagram of the worm nervous system is available
- Fully sequenced, more than 50% of worm genes have human homologs

C.ELEGANS AS A MODEL FOR DOPAMINERGIC DEGENERATION AND PARKINSON'S DISEASE

C. elegans: a relevant and useful model for PD

- For studying the central nervous system*:*

more than 50% of mammalian genes have orthologs in *C. elegans*

many of the same neurotransmitters as in mammals
302 neurons (vs.1 000 000 000 000 in humans)
all neuronal connections have been determined

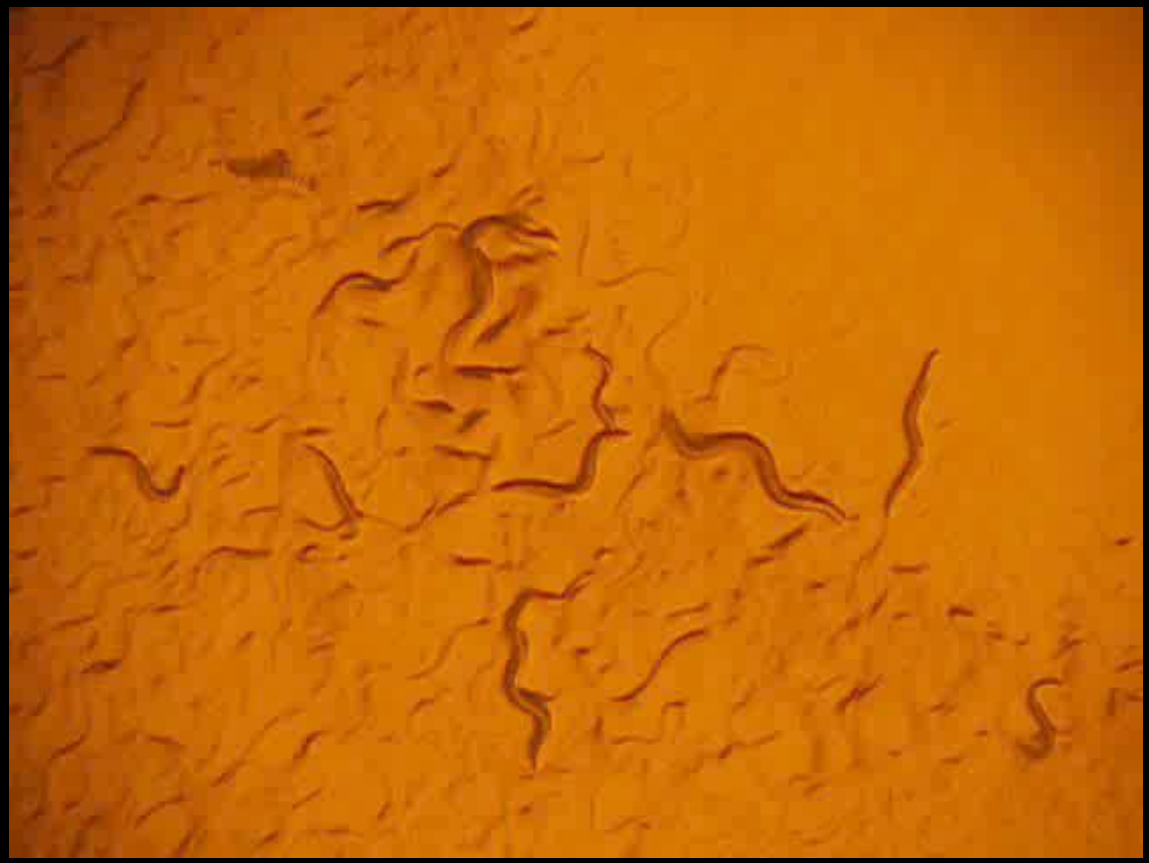
- For Parkinson's Disease:

similar dopamine metabolism
conserved enzymes and transporters
8 dopaminergic neurons in hermaphrodites
how does *C. elegans* PD look like?

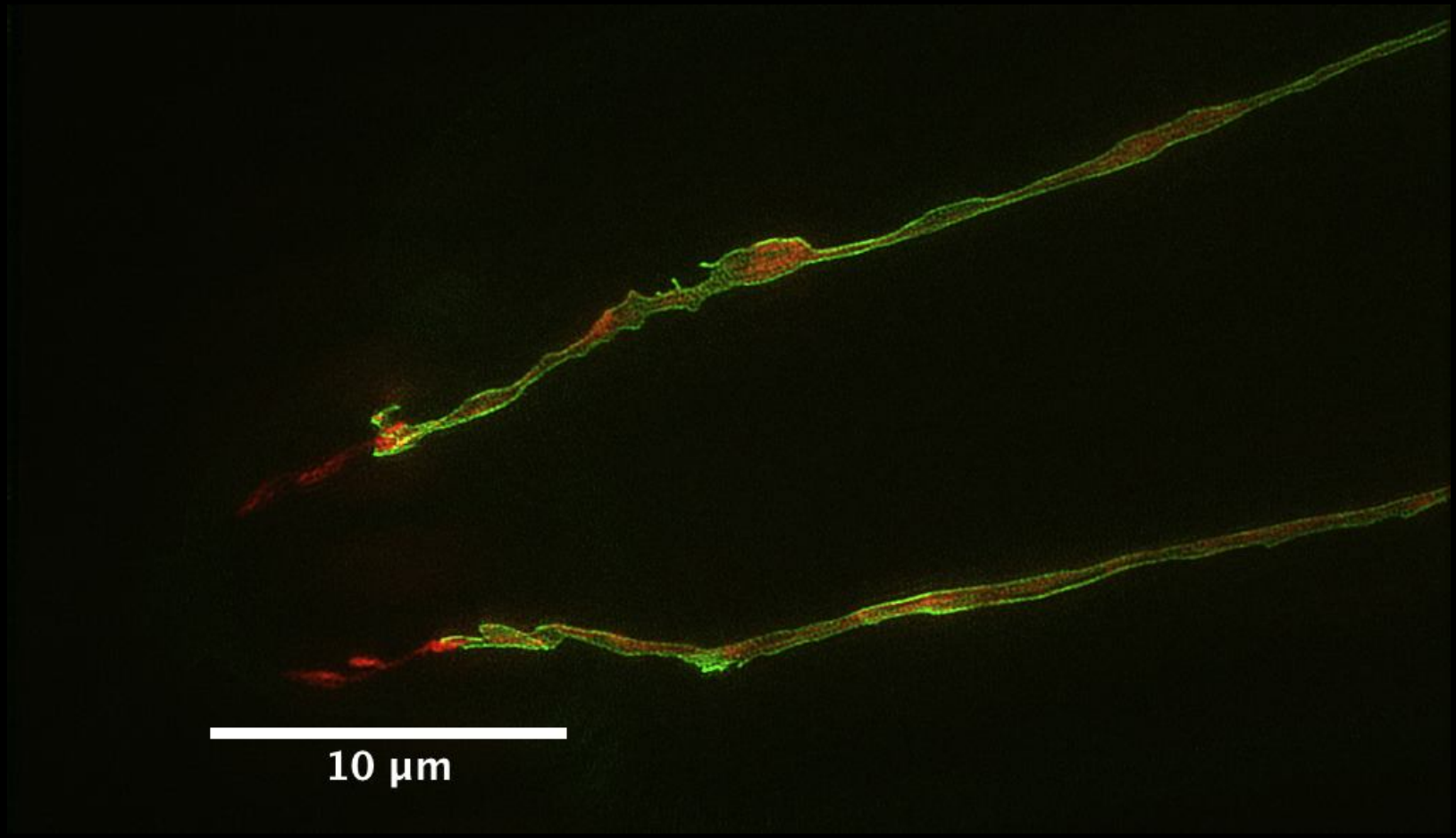
- To mimic dopaminergic death:

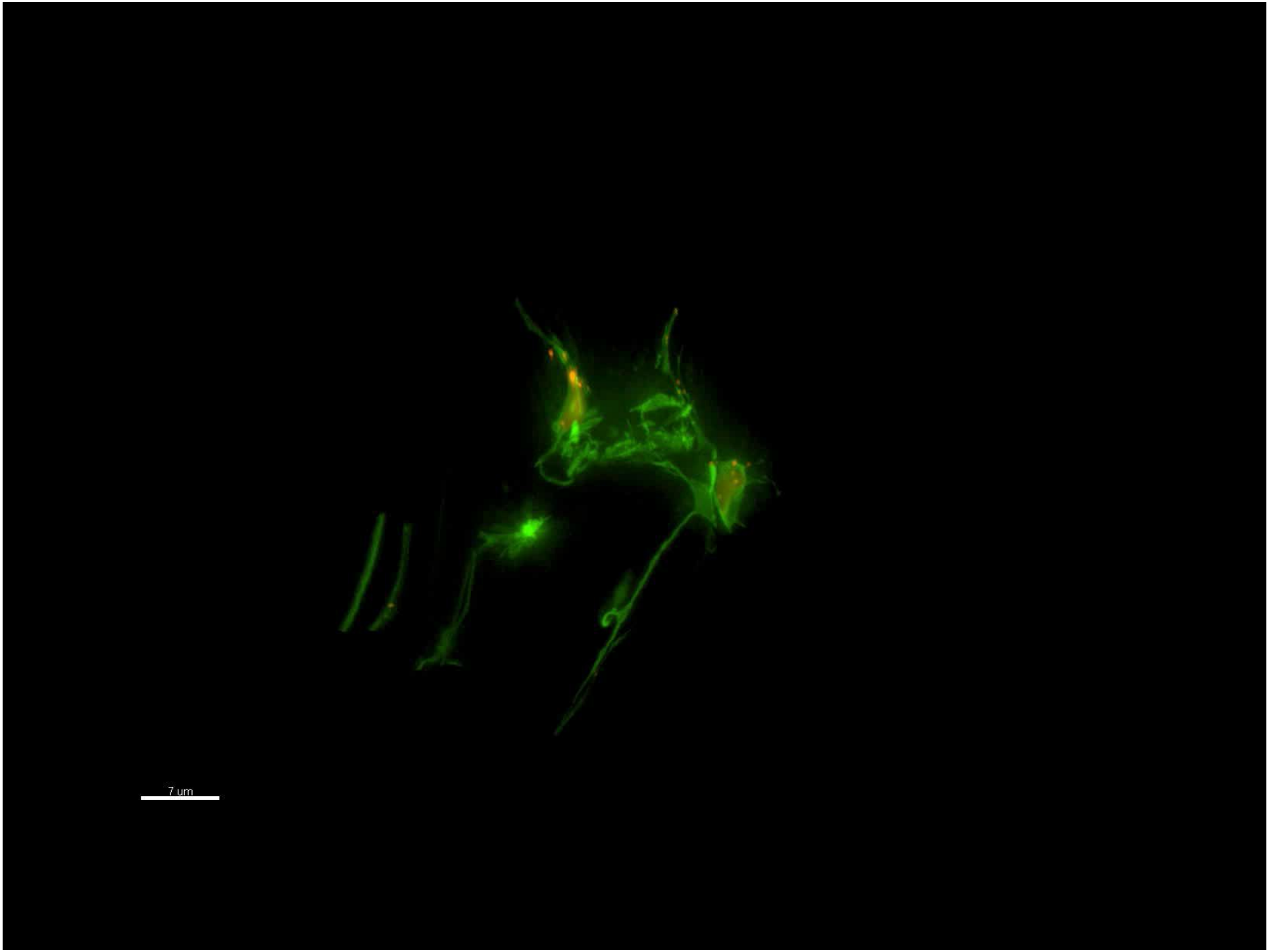
conservation of genes involved in PD
sensitivity and specificity of toxin

C. ELEGANS



Dendritic part of C. elegans dopaminegic neurons

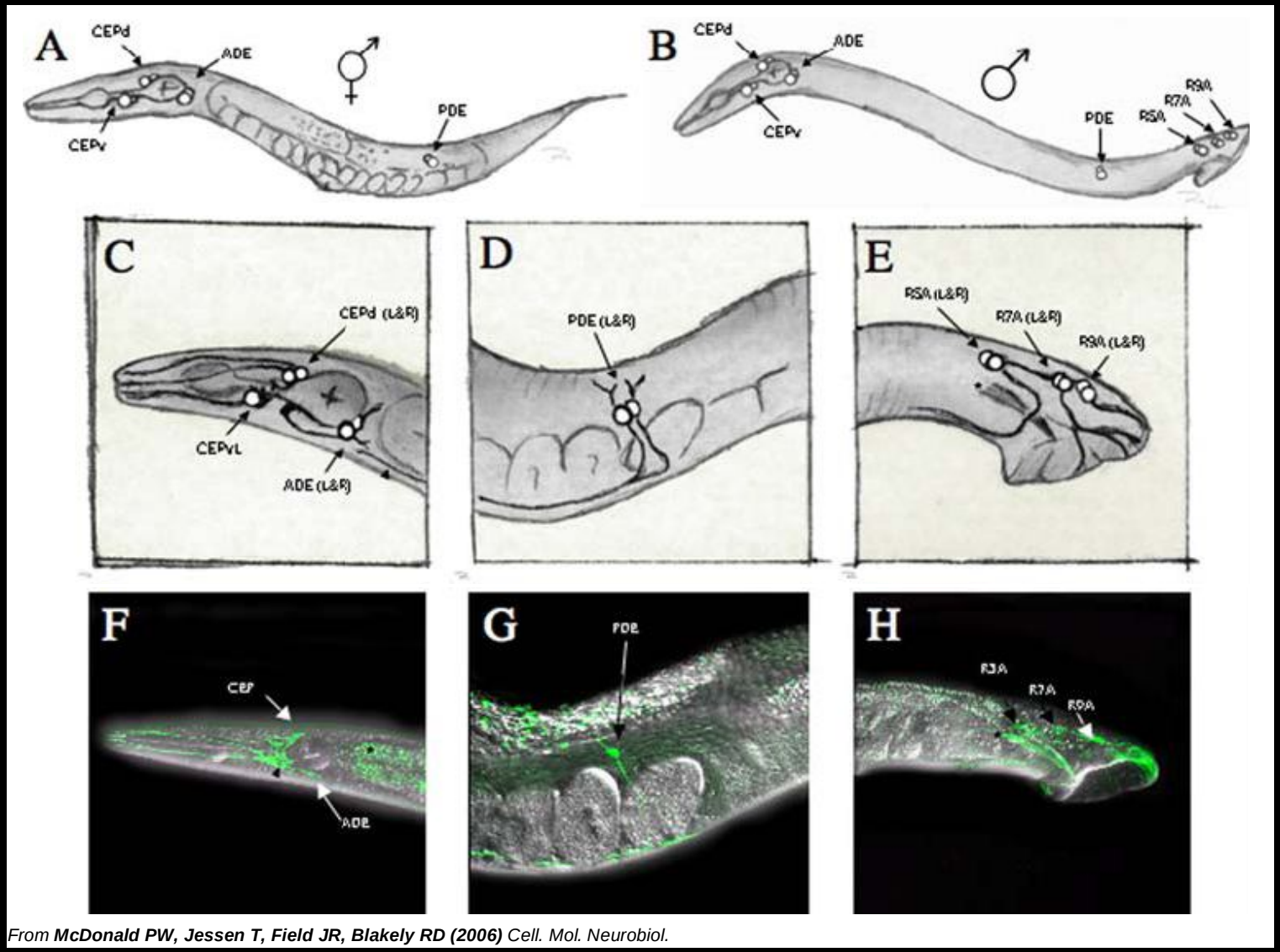



7 um

Most human PD genes are conserved in C. elegans

Gene	*C.elegans* gene		Strain	Function
SNCA	not conserved, overexpression phenotype in worms		none	unknown
PARKIN	***pdr-1*/**K08E3.7	1e-48	**tm0598**	E3 ligase
UCHL1	***ubh-1*/**F46E10.8	1.7e-30	**tm0526**	Ubiquitin hydrolase
	***ubh-3*/Y40G12A.1**	4.5e-28	tm2550	
	***ubh-2*/Y40G12A.2**	2.6e-25	tm0526	
PINK1	***pink-1*/**EEED8.9	2.7e-53	**tm1779**	Kinase
DJ1	***djr-1.1*/**B0432.2	3e-46	**tm0918**	Oxidative stress
	***djr-1.2*/C49G7.11**	6e-35	**tm1346**	
LRRK2	***lrk-1*/T27C10.6**	2.2e-67	**tm1898**	Kinase

BY200: a strain with GFP dopaminergic neurons



From ***McDonald PW, Jessen T, Field JR, Blakely RD (2006)*** *Cell. Mol. Neurobiol.*

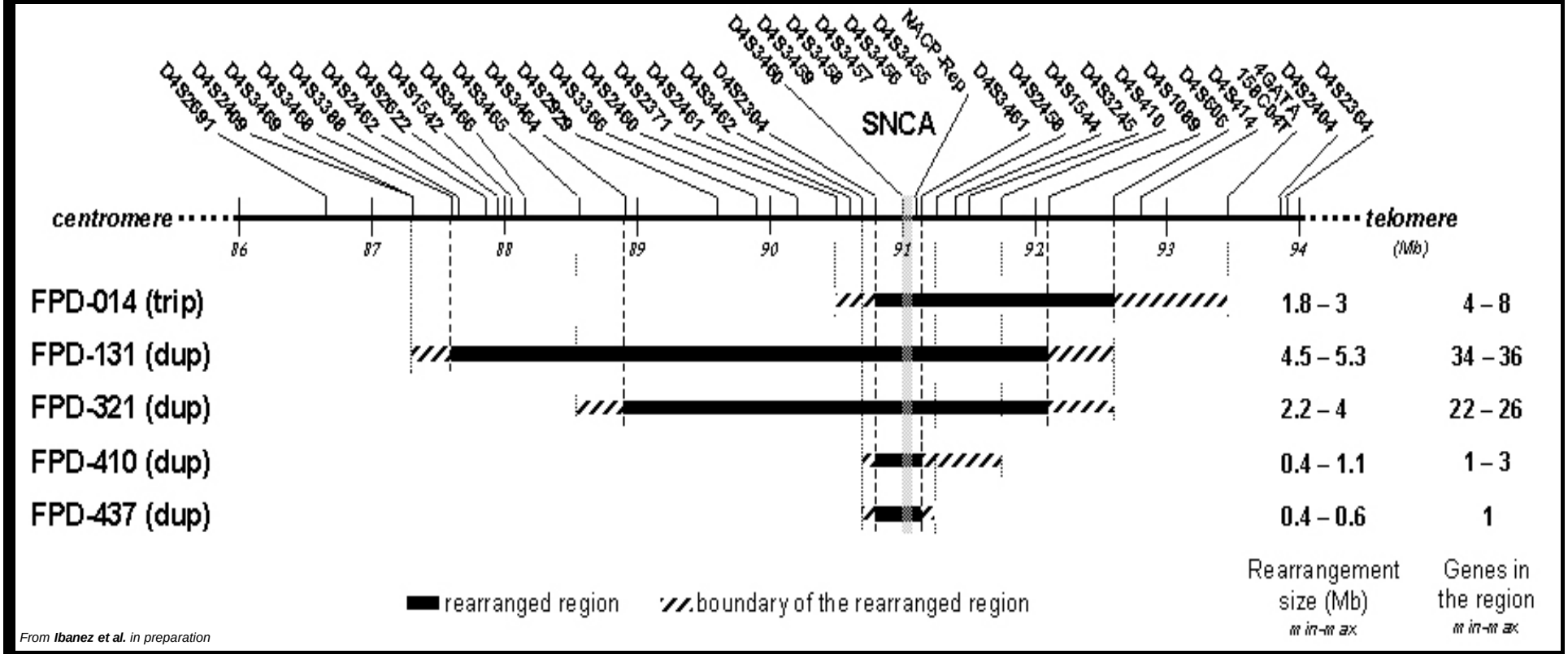
assay dopaminergic degeneration

Nass et al. (2002) *Proc. Natl. Acad. Sci. U S A*

Mutations in the *α-SYNUCLEIN* Gene

Locus	Inheritance	Clinic	Onset age	Gene	Reference
PARK1	Dominant	± dementia	40s	α-SYNUCLEIN	*Polymeropoulos 1997*
PARK2	Recessive	Slow progression	20-40	PARKIN	*Kitada 1998*
PARK3	Dominant	typical	60s	?	*Gasser 1998*
PARK4	Dominant	a/typical	40/60	α-SYNUCLEIN	*Singleton 2003*
PARK5	Dominant	ty		UCH-L1	*Leroy 1998*
PARK6	Recessive	Slow pr		PINK1	*Valente 2004*
PARK7	Recessive	Slow pr		DJ-1	*Bonifati 2003*
PARK8	Dominant	± de		LRRK2	*Paisan-Ruiz 2004*
PARK9	Recessive	Kufo		ATP13A2	*Ramirez 2006*
PARK10	Dominant	ty		?	*Hicks 2002*
PARK11	Susceptibility	ty		?	*Pankratz 2002*
PARK12	Susceptibility	ty		?	*Pankratz 2002*

Increased Severity with *SNCA* Copy Number



From ***Ibanez et al.*** *in preparation*

DOSE EFFECT:

TRIPLICATION – diffuse Lewy Body disease

DUPLICATIONS – typical PD

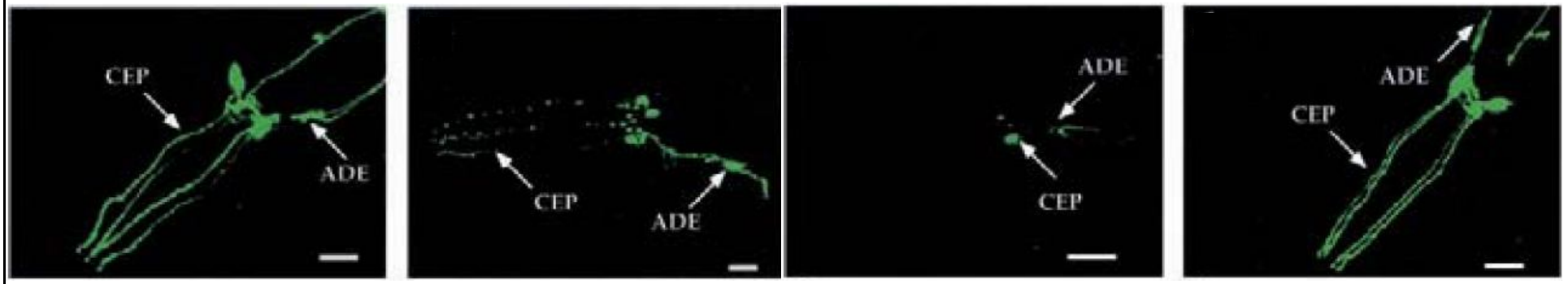
Ibanez et al. (2004) *Lancet*

6-OHDA: a toxin for dopaminergic death

- **BY200**

6-OHDA

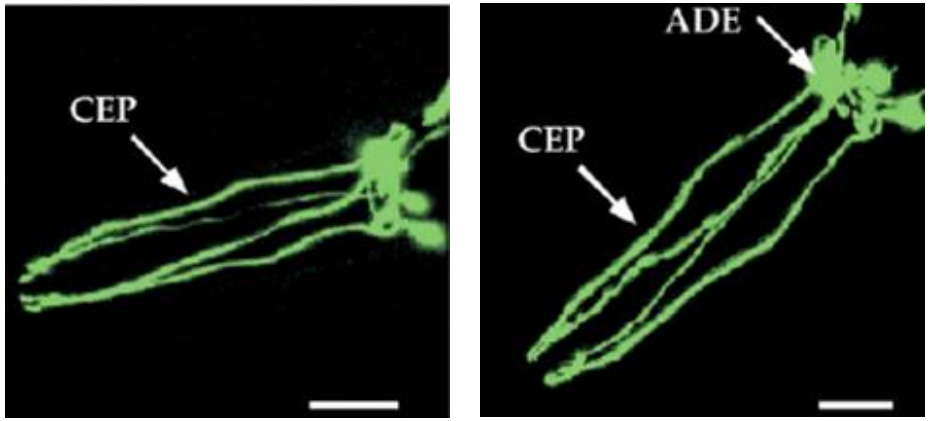
+ imipramine

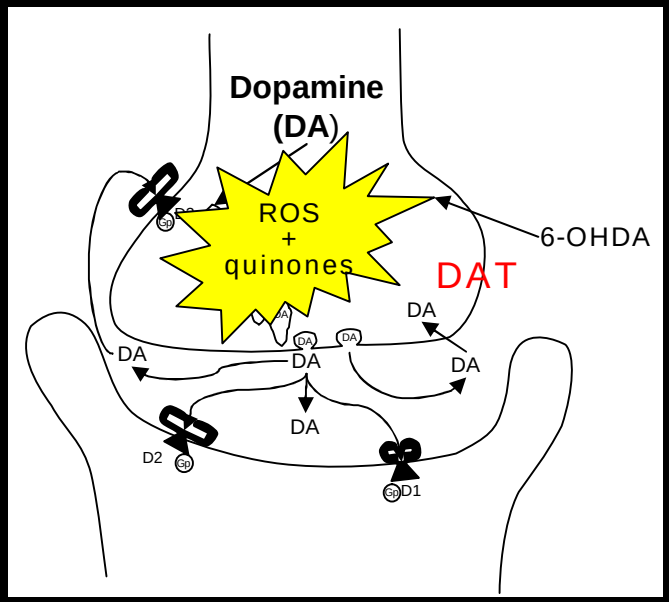


Nass et al. (2002) *Proc. Natl. Acad. Sci. U S A*

- **BY200 x ΔDAT-1**

6-OHDA





Nass and Blakely (2003) *Annu. Rev. Pharmacol. Toxicol.*

Studying *PINK1* kinase in *C.Elegans*

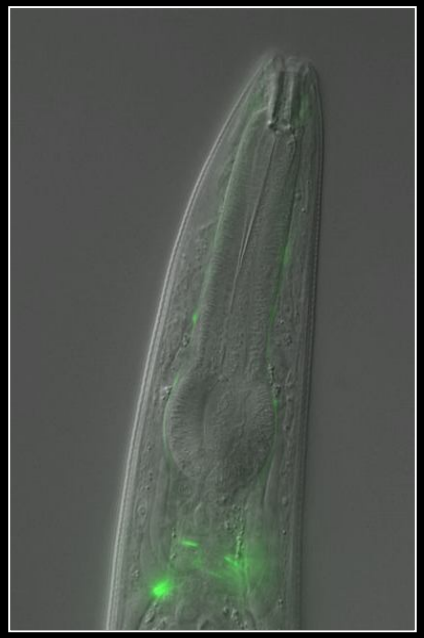
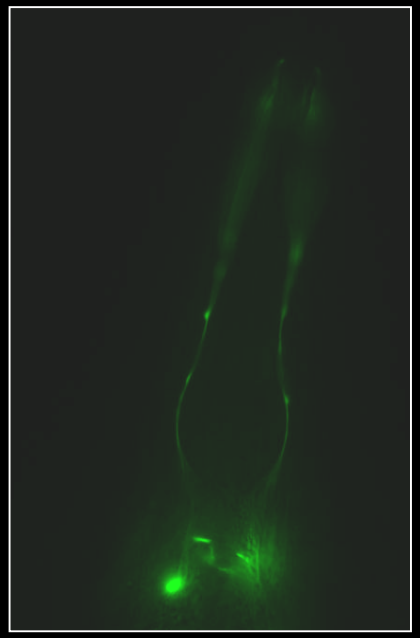
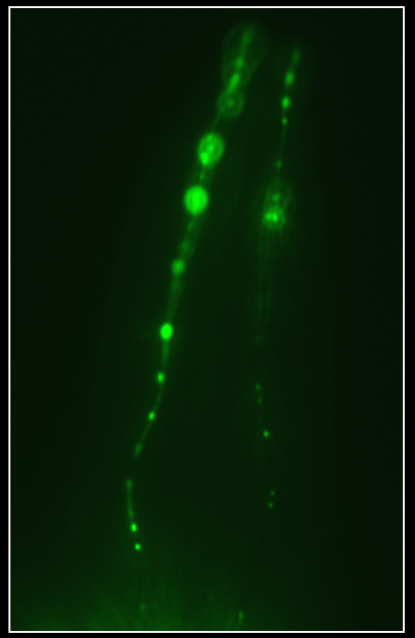
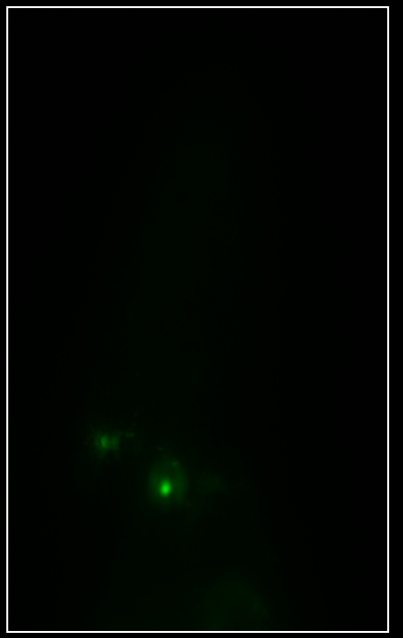
PINK1: kinase genes
pink-1(tm1779) *C.Elegans* strains

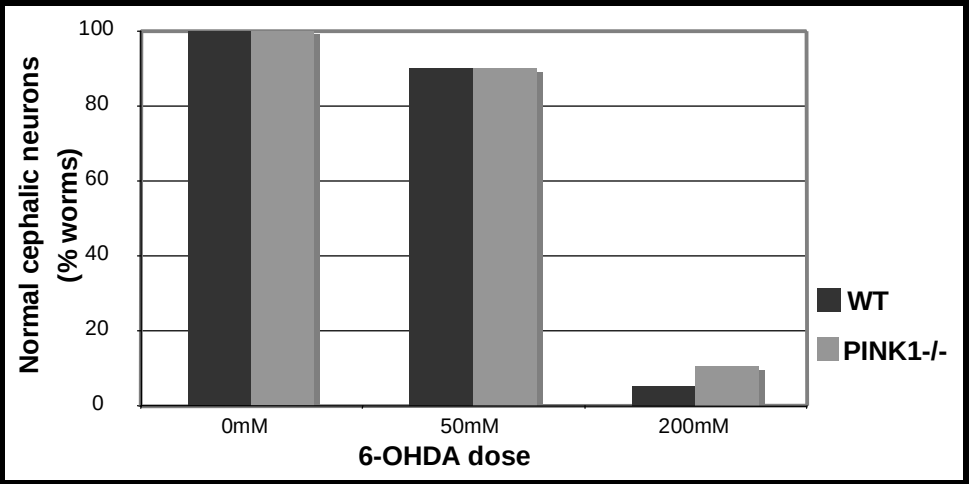
-are gene deletions in C.elegans associated with any phenotype?

-do these genes protect dopaminergic neurons?

No overt phenotype upon 6-OHDA intoxication

6-OHDA



- preliminary results indicate no overt increase in 6-OHDA mediated neurodegeneration in pink-1(PINK1) and lrk-1 (LRRK2) knockouts
- pink-1/lrk-1 strains have to be tested

Louise Chapman

Neurotoxic intoxication to reveal susceptibility

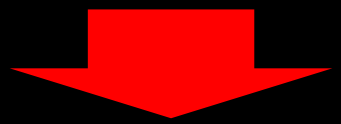
Challenge worms with dopaminergic neurotoxin regimes:

6-OHDA (Nass *et al.*, 2002)

1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)

Rotenone

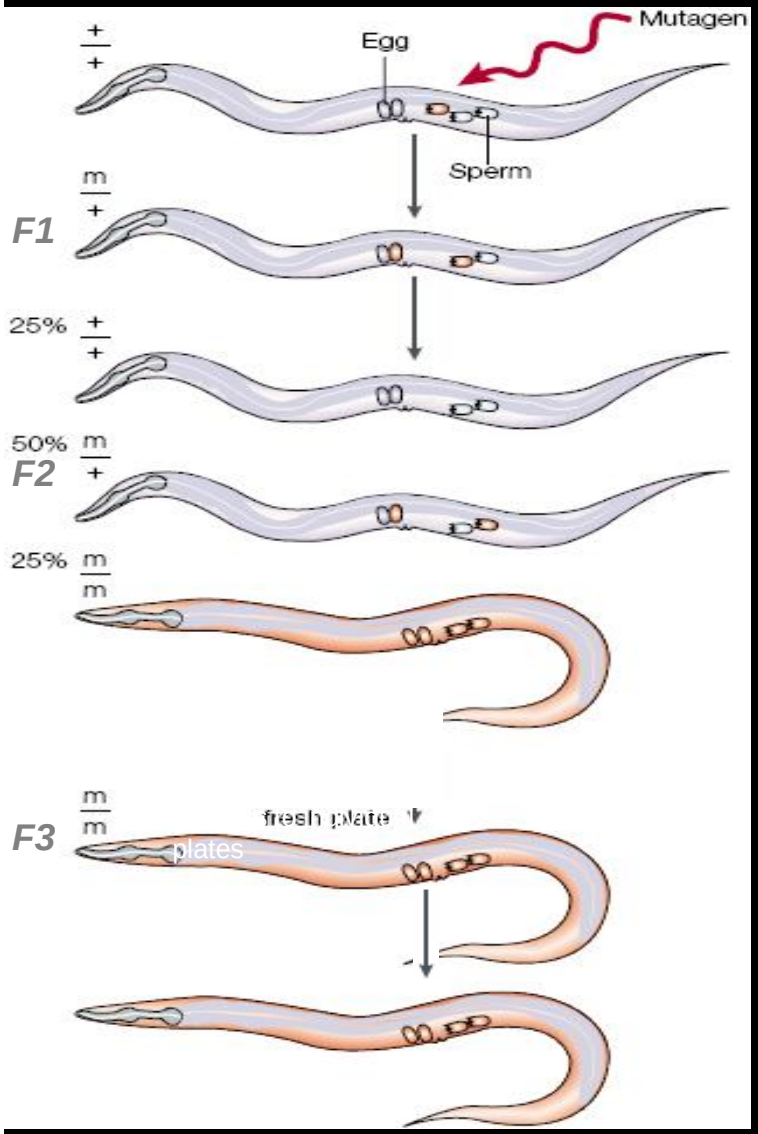
Paraquat



Look for factors (genes) that lead to enhanced or suppressed neurodegeneration

- *Genetic screen for hypersensitivity to dopaminergic toxins*

Screening for 6-OHDA Hypersensitivity or Resistance



P*dat-1*::GFP worms mutagenesis

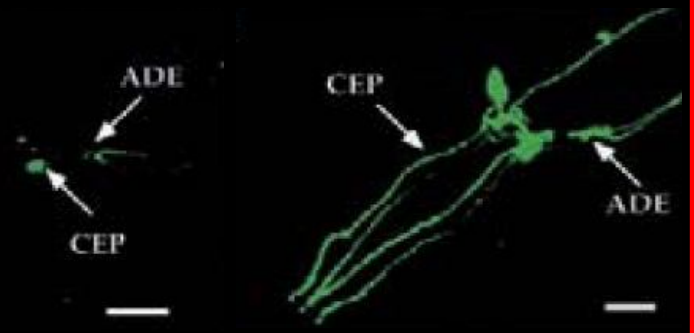
6-OHDA intoxication

Screen under low magnification fluorescence microscope for candidate mutants

Isolation, phenotype confirmation in subsequent generation and after back crosses

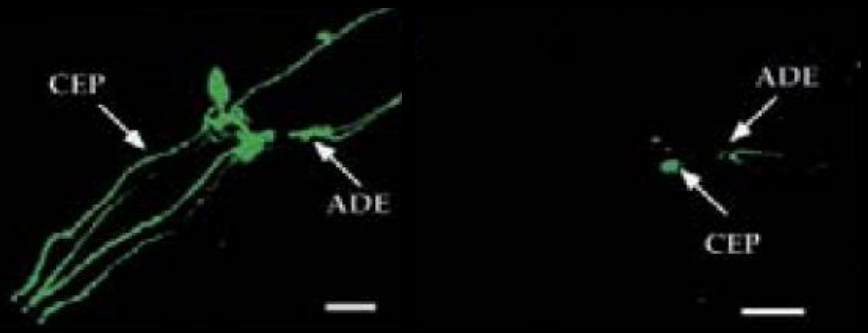
6-OHDA Resistance and Hypersensitivity Screens

Highly toxic 6-OHDA doses (50mM):



Wild type **Resistant**

Non-toxic 6-OHDA doses (10mM):



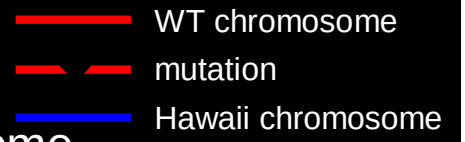
Wild type **Hypersensitive**

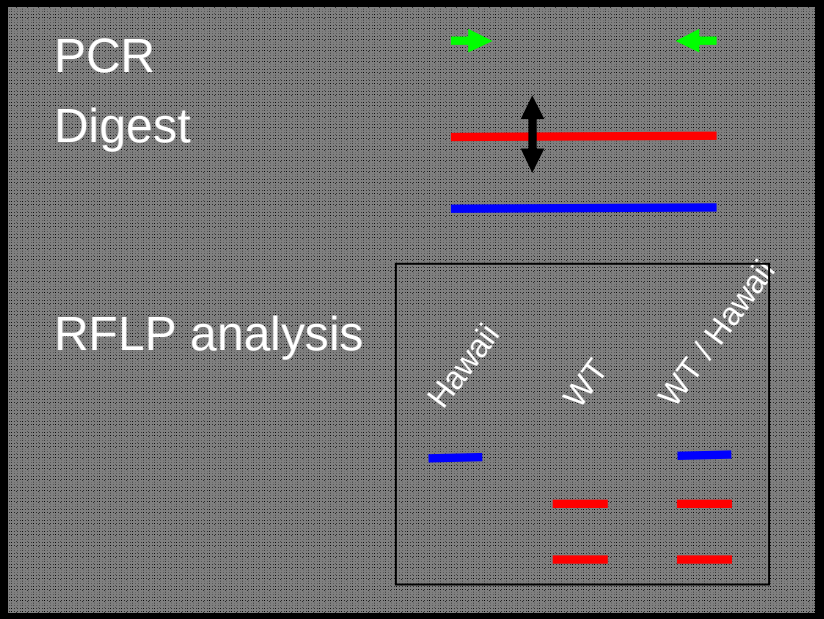
	Resistant				Hypersensitive			
Dose (mM)	300	250	200	50	10	5	2.5	1.25
Intoxicated (~)	600	600	600	2153	2400	2400	2776	2608
Screened	4	-	103	1487	2216	2093	2052	1823
Phenotype	1	0	0	11	64	25	14	4
Confirmation	0	0	0	**2**	**1**	0	0	0

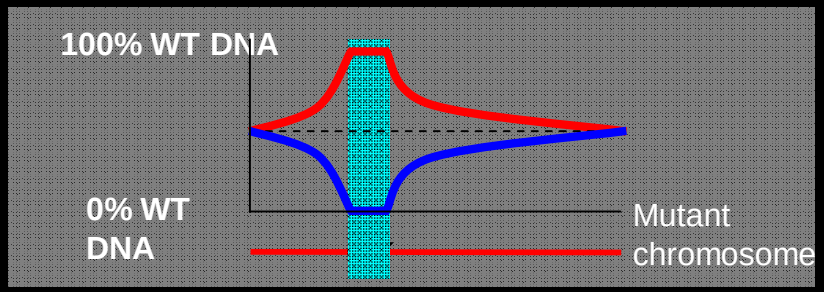
Positional Cloning By SNP Mapping

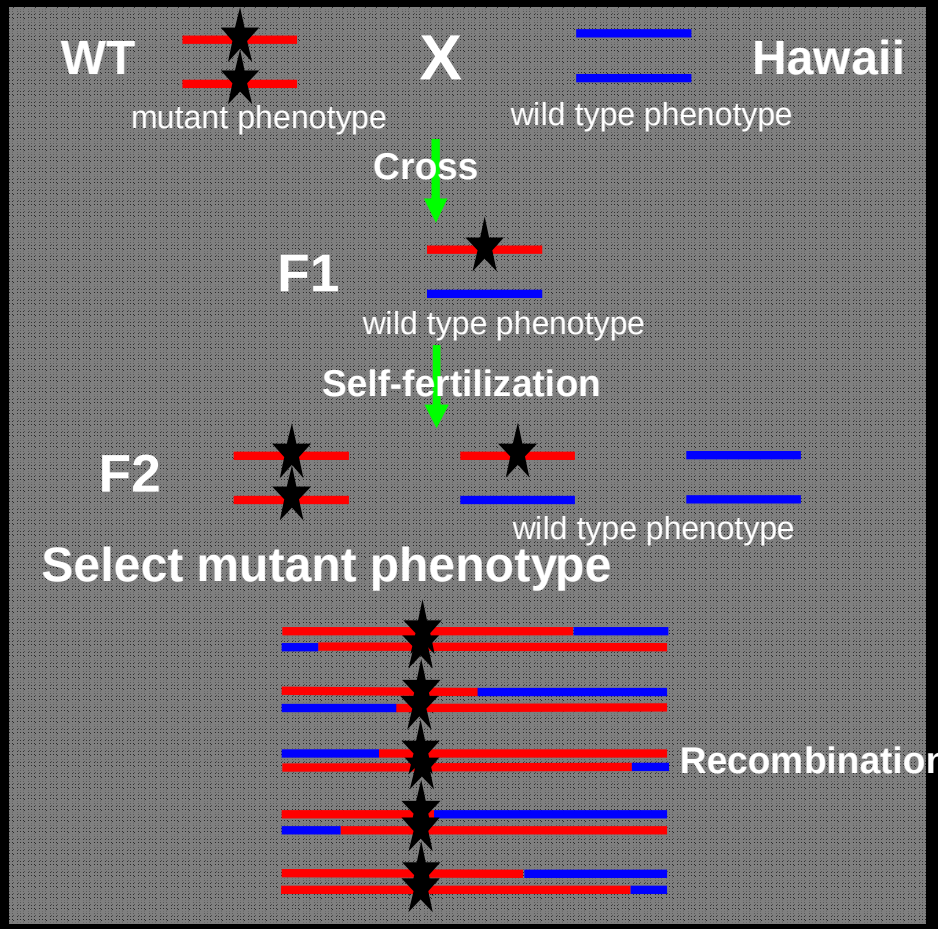
Mutagenised P*dat-1*::GFP worms are on N2 back ground

Back crossing aims at reducing the number of mutations in the genome

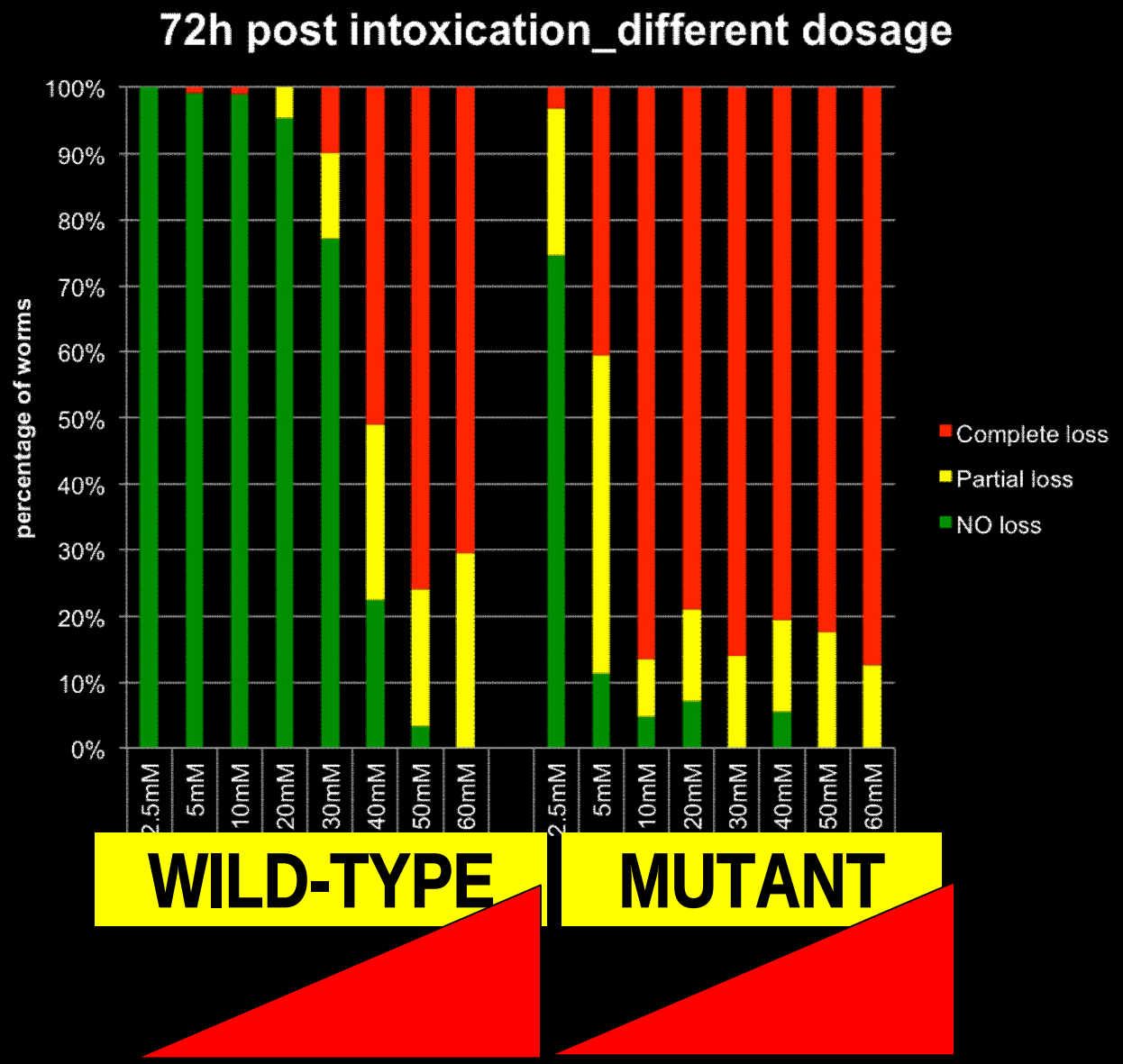




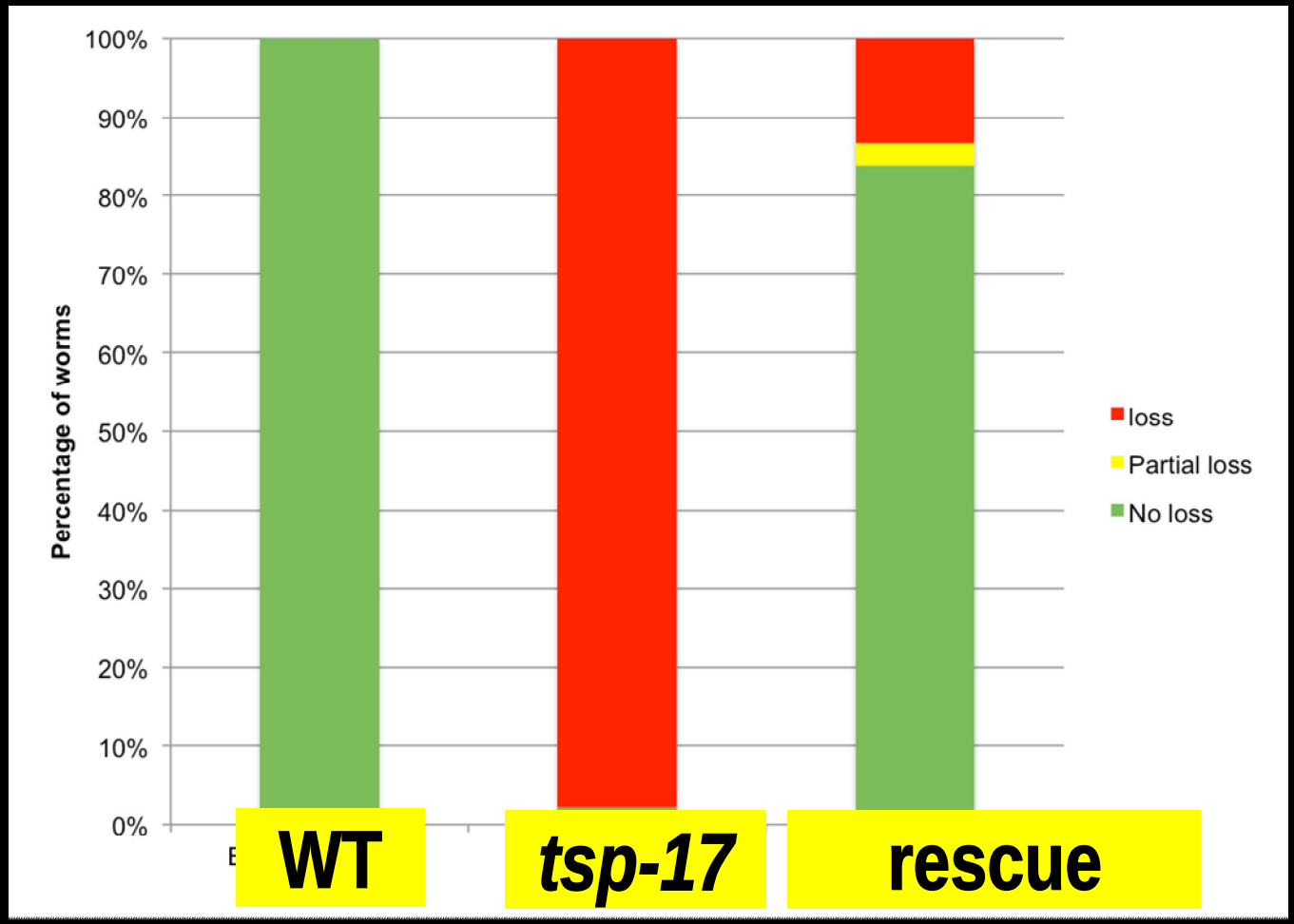




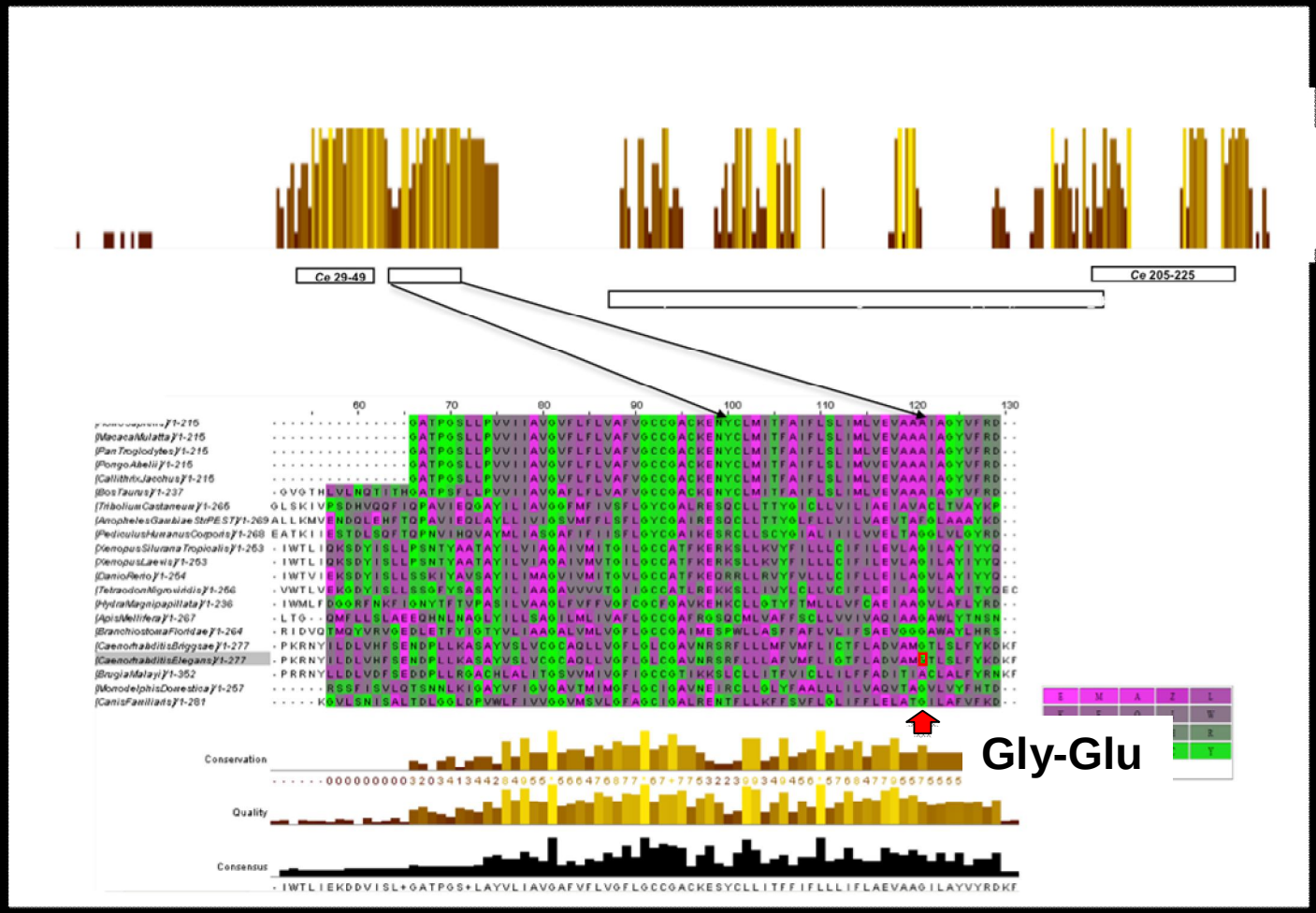
The *gt1681* Mutant Is Hypersensitive to 6-OHDA (Loss of Dopaminergic Neurons)

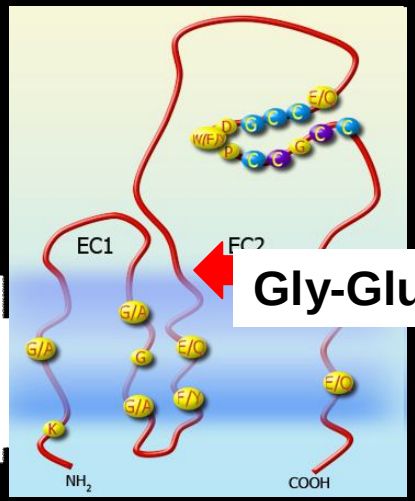


Rescue of the *tg1681* 6-OHDA Hypersensitivity by a TSP-17 Encoding Fosmid



Alignment of *C.elegans tsp-17* with Orthologues





Where does TSP-17 act?

Evidence for TSP-17 acting in dopaminergic neurons

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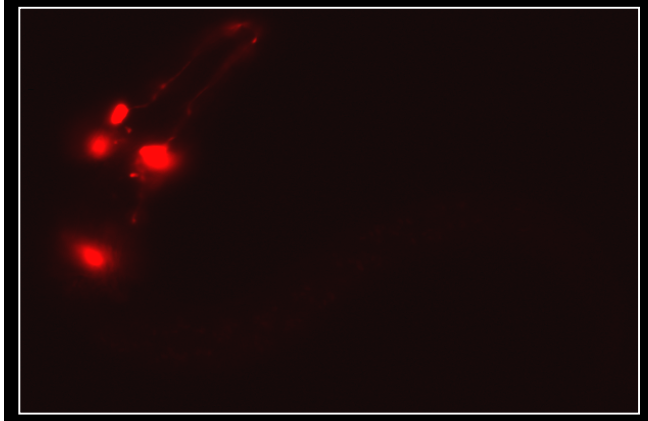
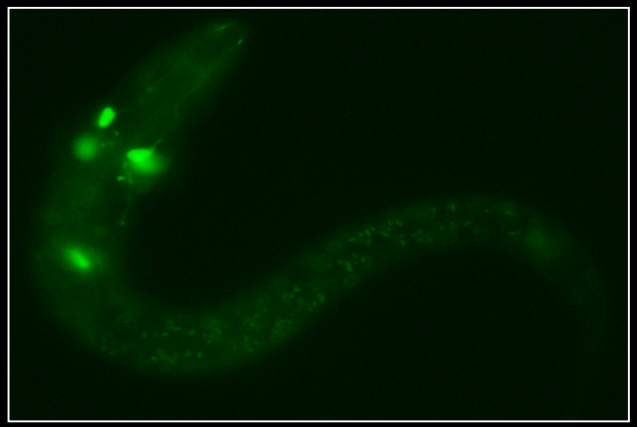
Protein Phosphorylation Unit

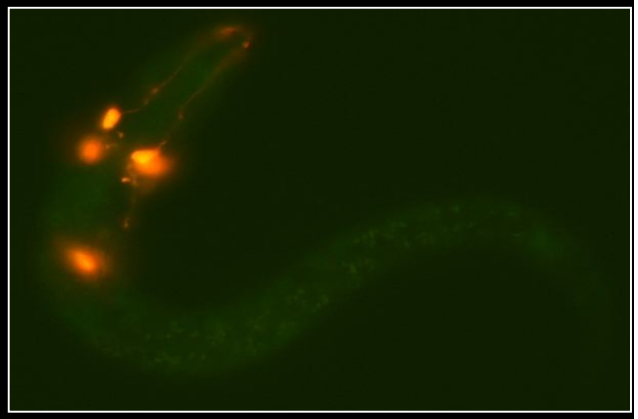
tsp-17 is expressed in the dopaminergic neurons



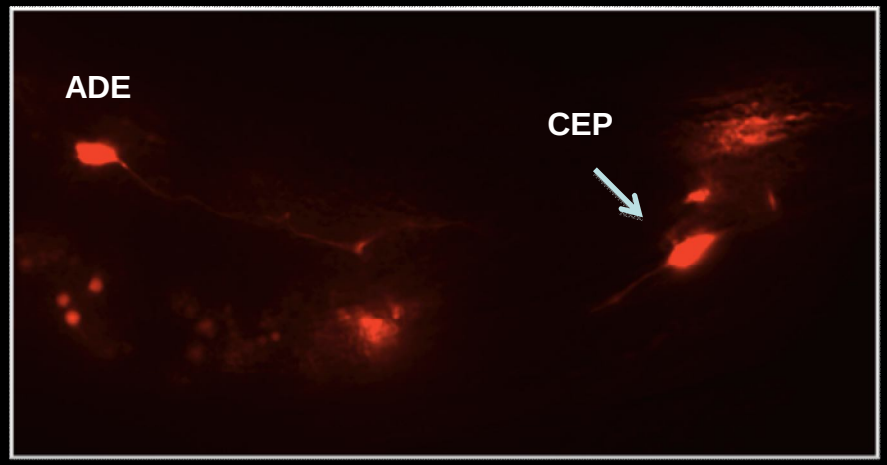
Pdat-1:: yfp:: 3'let858



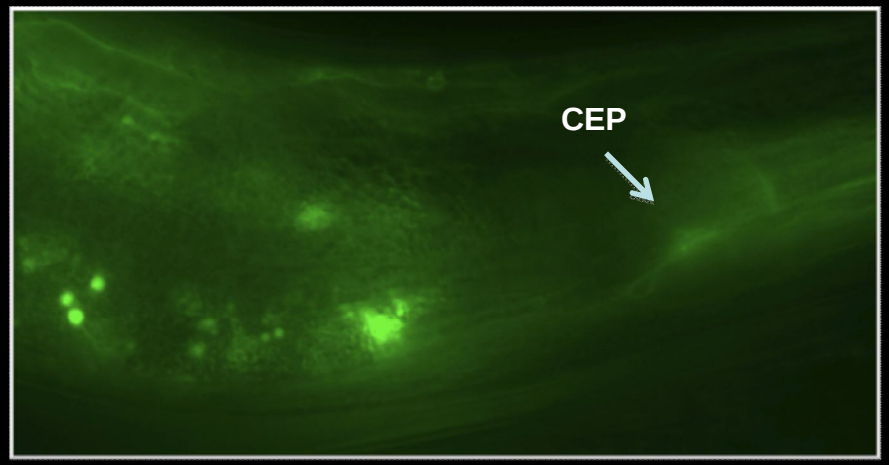
Ptsp-17:: tsp-17::cfp:: 3'tsp-17



merge



pdat-1:: mcherry:: 3'let858



ptsp-17:: tsp-17::gfp:: 3'tsp-17

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