MY RETURN TO CELL REPLACEMENT: WHAT IS THE PROMISE WHAT ARE THE CHALLENGES

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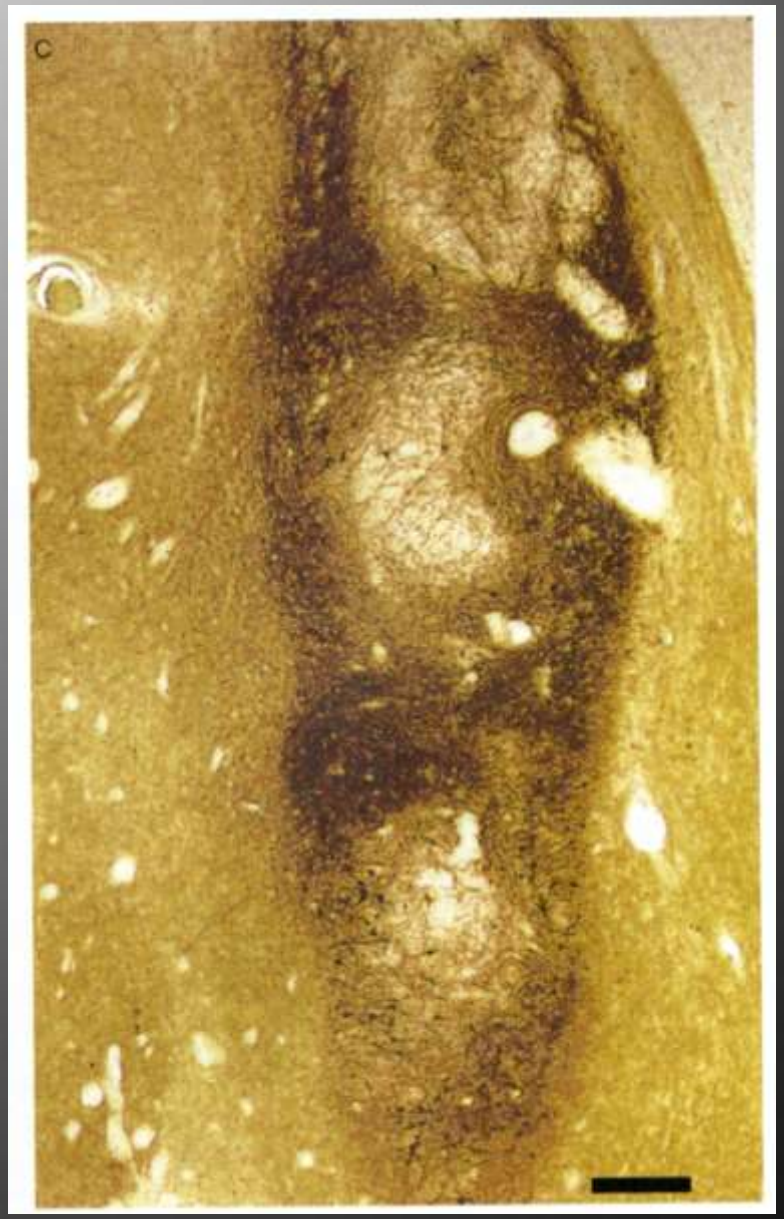
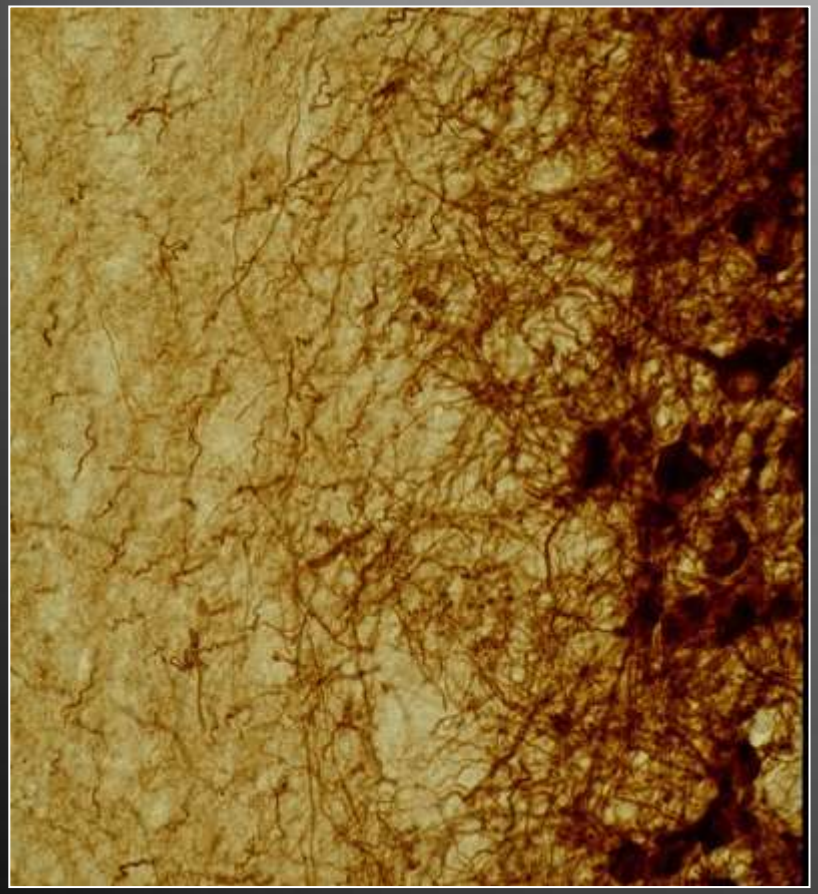
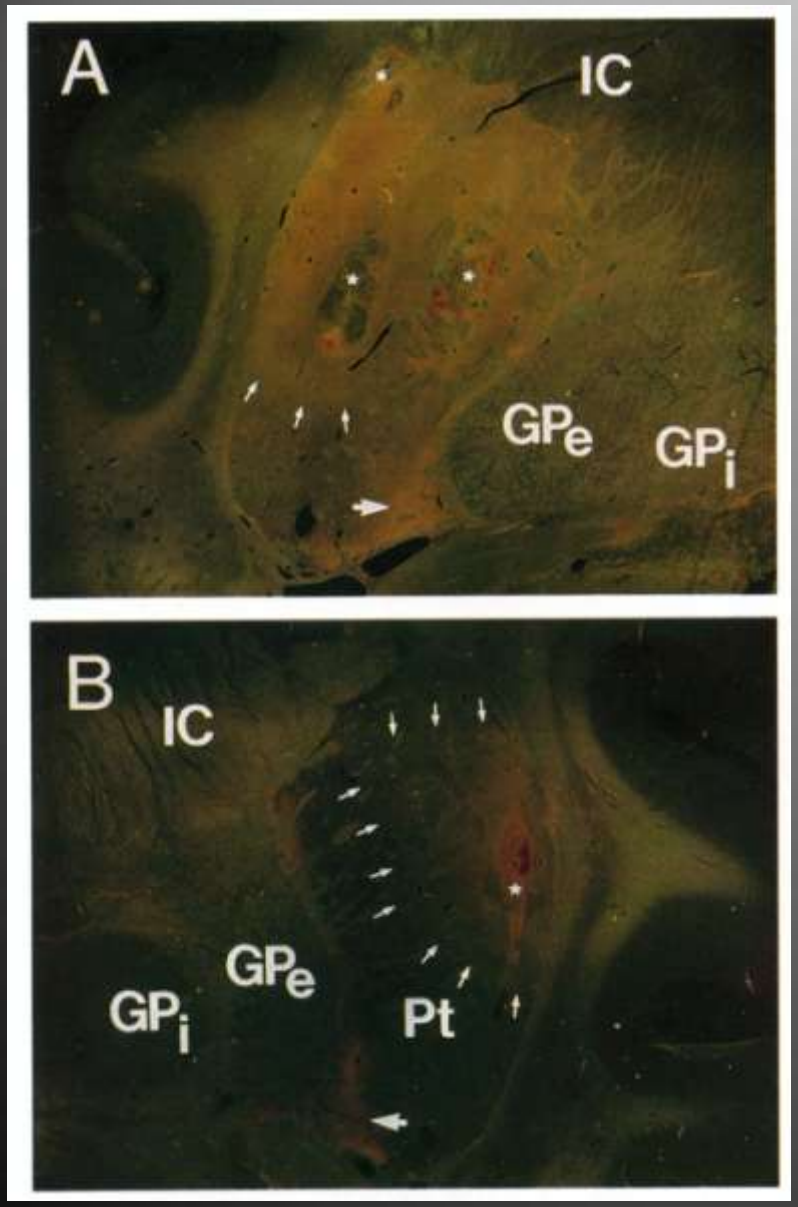
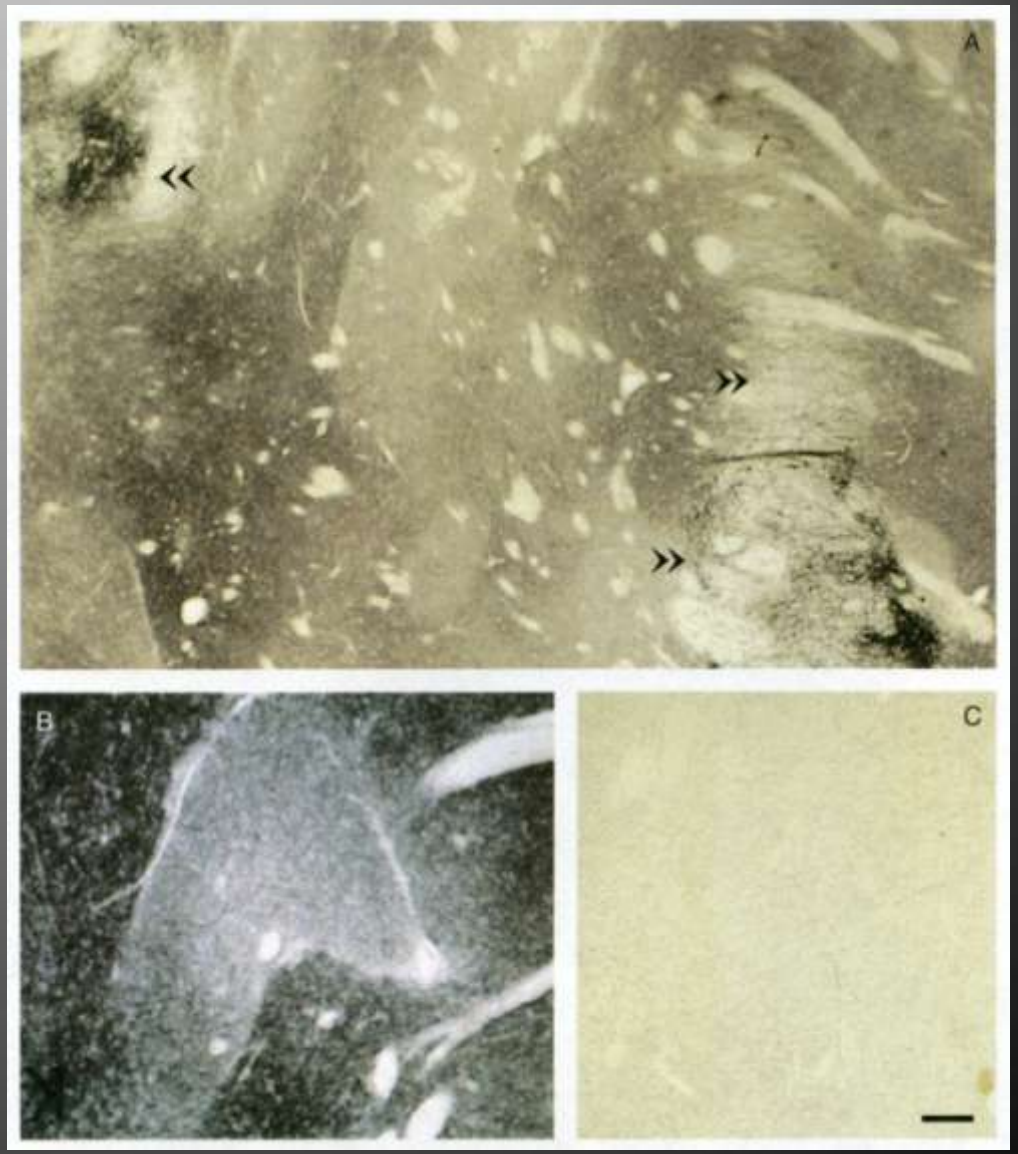
B


C

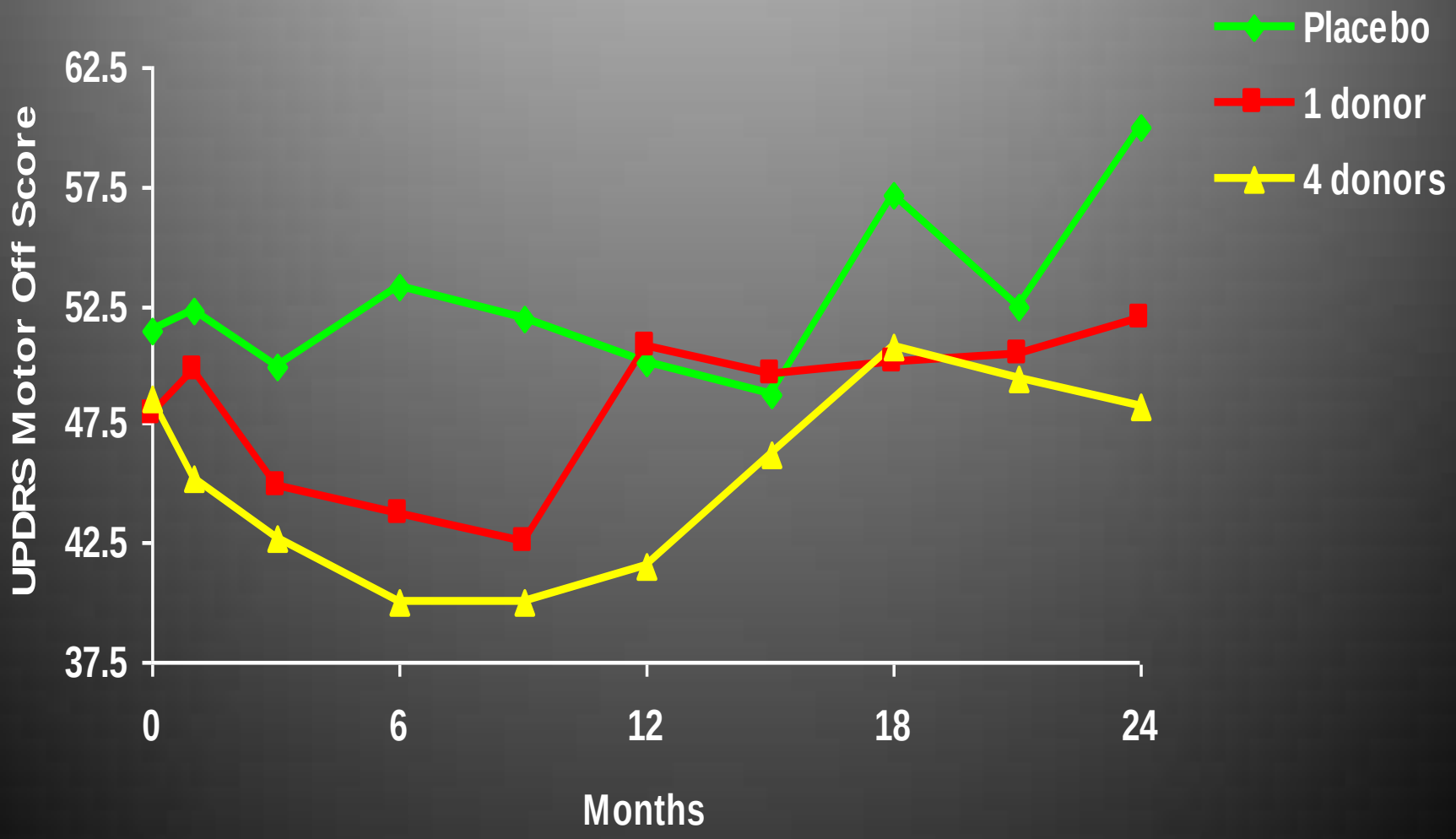
Fetal Nigral Transplantation
Graft-Striatal Interface and normal morphology

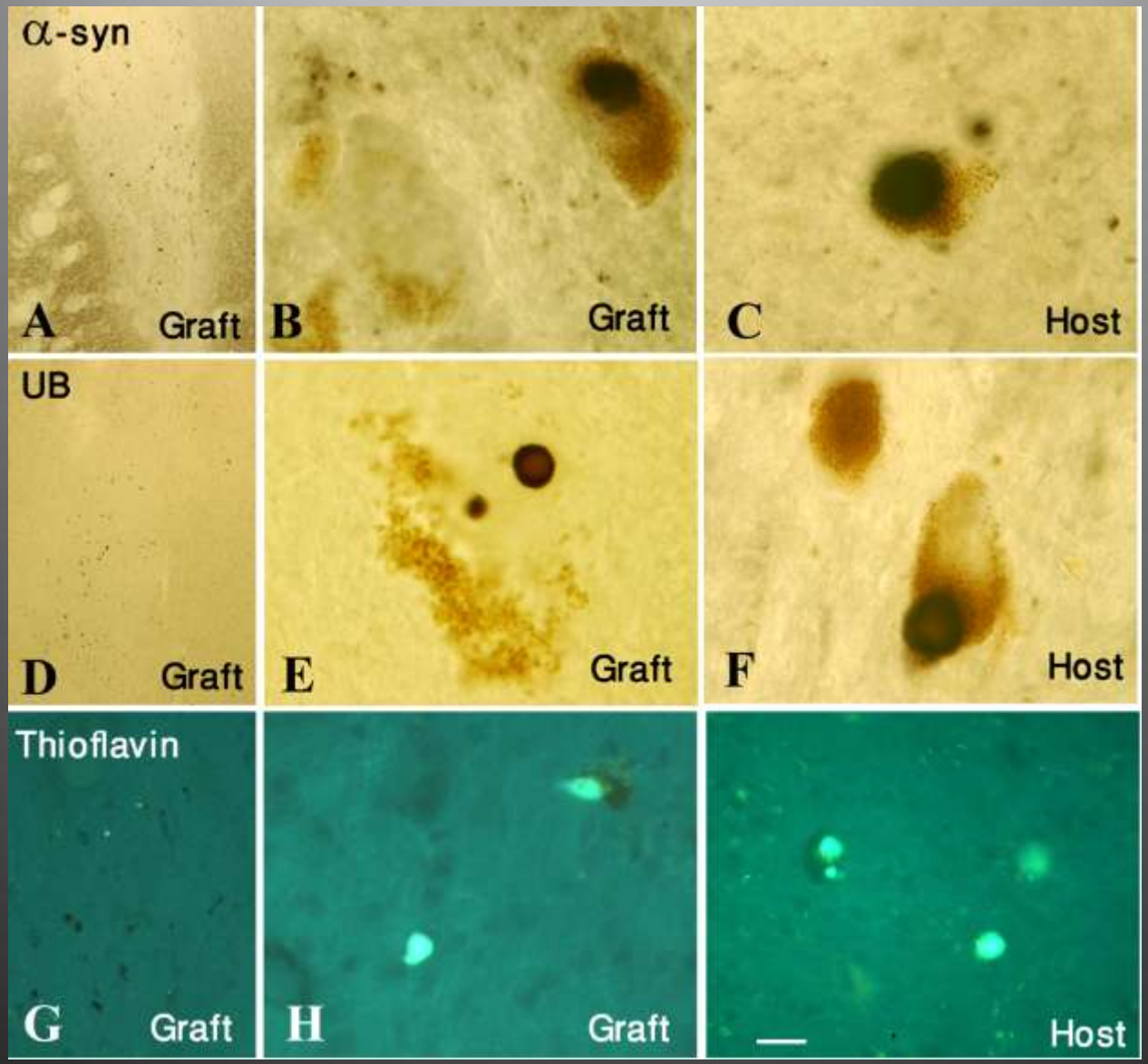



A
IC
GPe
GPi
B
IC
GPe
GPi
Pt

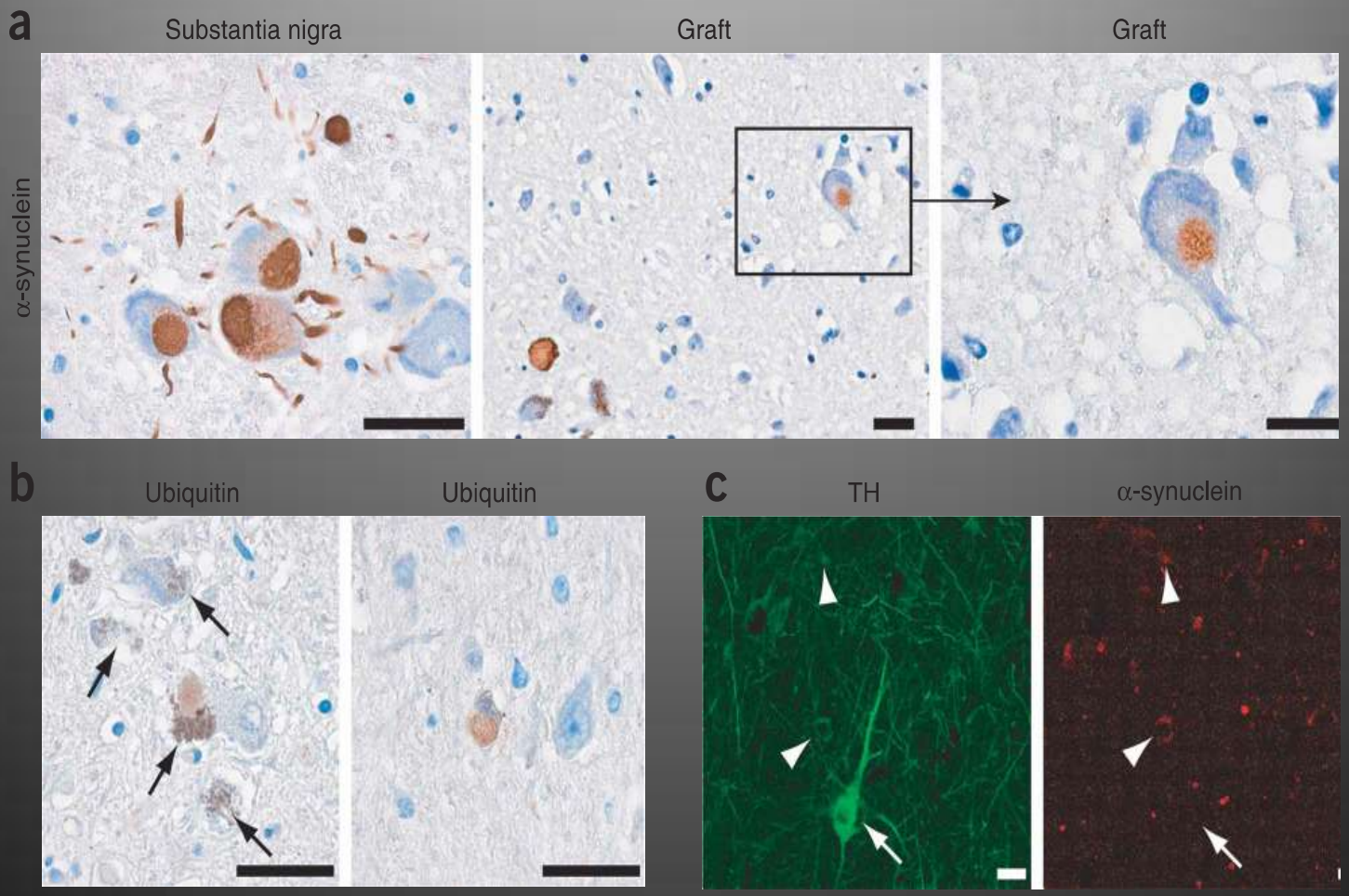

A
B
C

Fetal Nigral Transplant Study
Mean UPDRS Motor Off Score by Visit




α-syn
A
Graft
B
Graft
C
Host
UB
D
Graft
E
Graft
F
Host
Thioflavin
G
Graft
H
Graft
Host

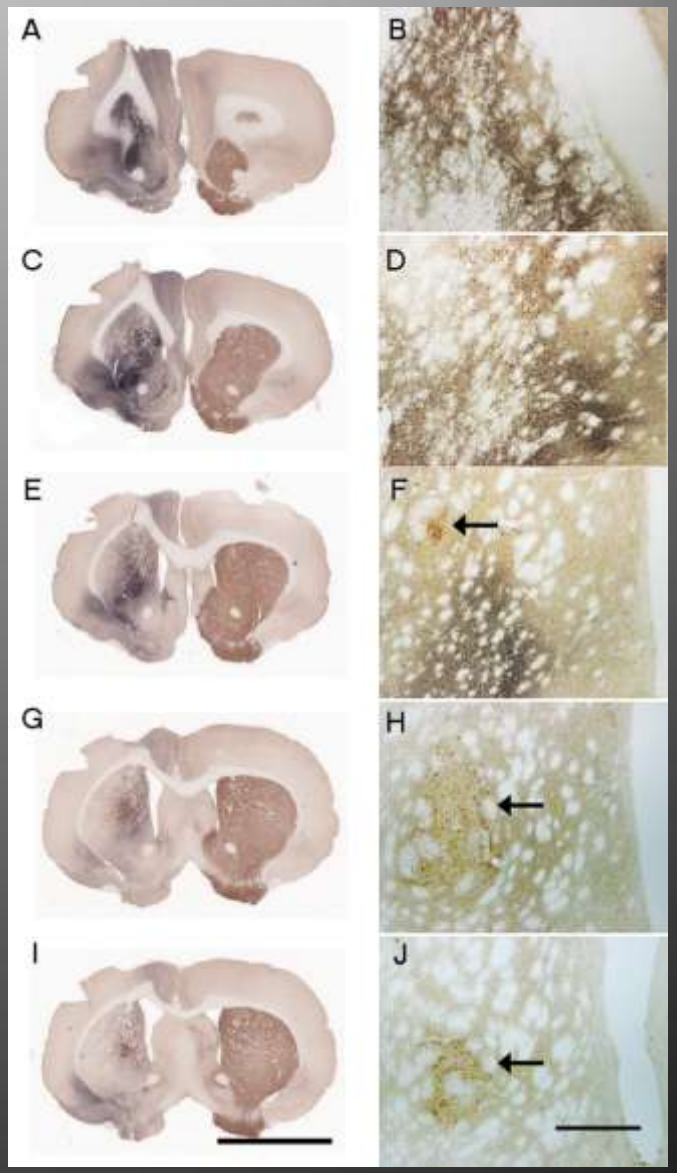
Who sees Lewy Bodies in Grafts? Everyone who really looks



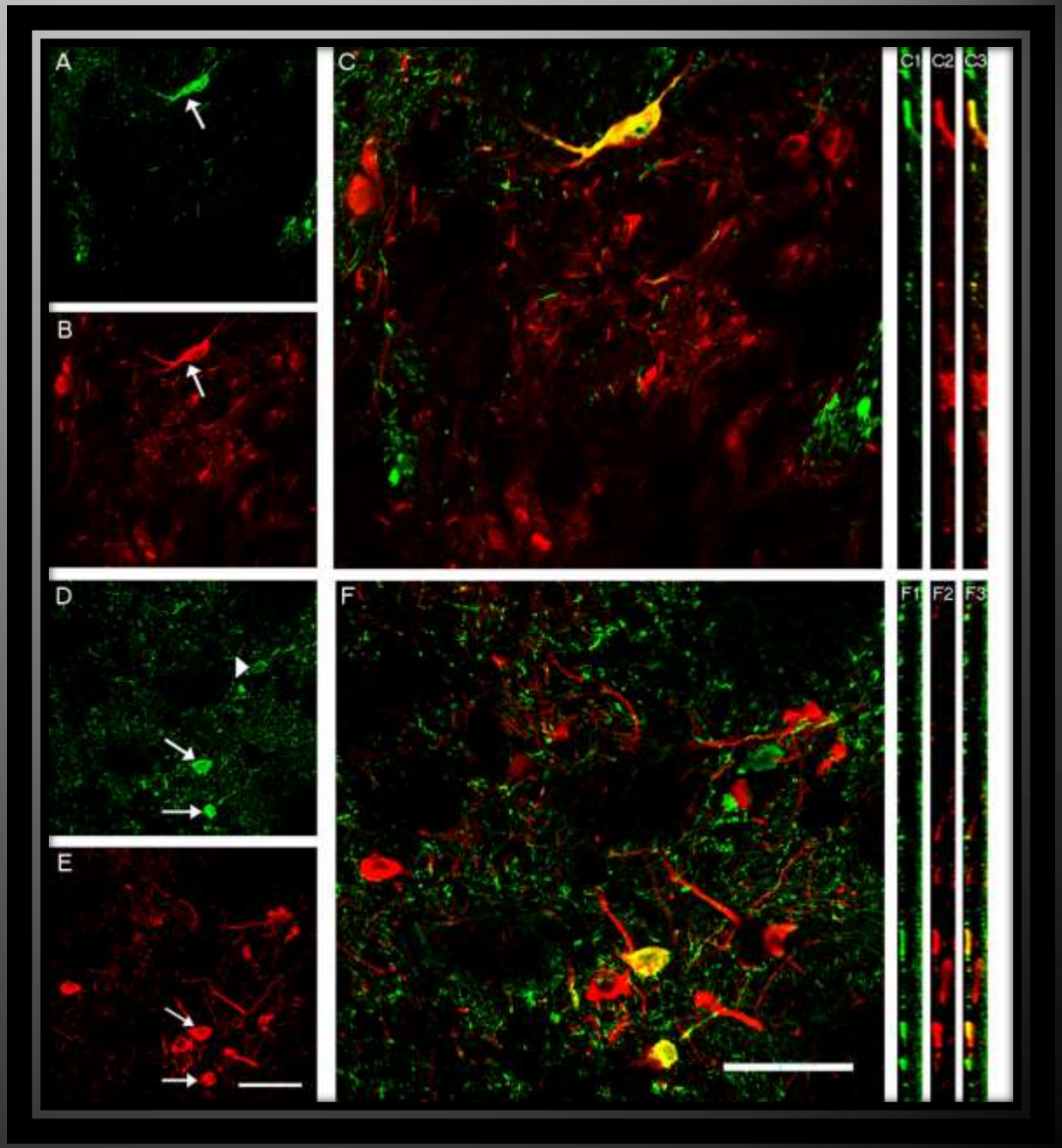
Li et a., Nature Medicine, 2009

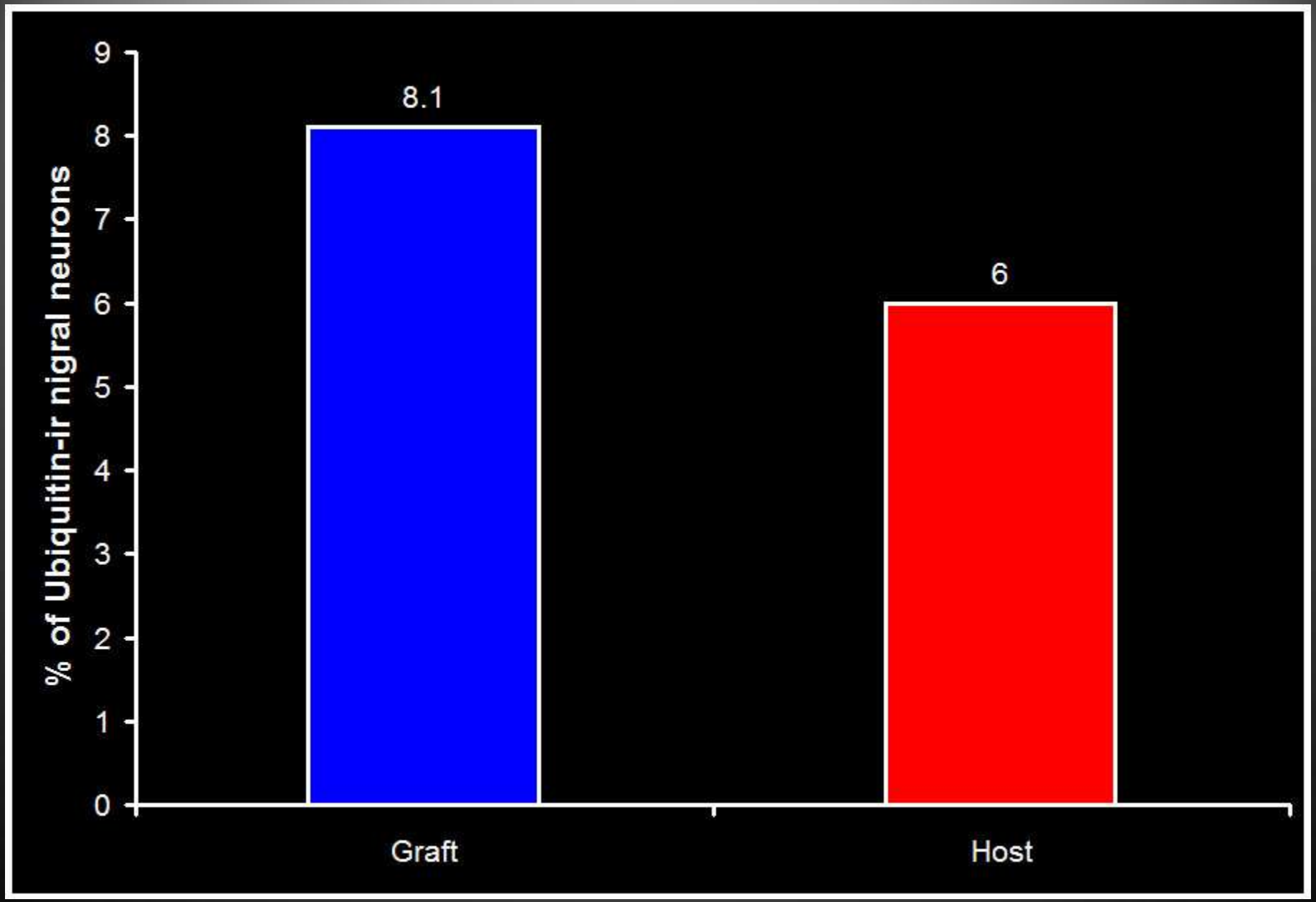
Grafts of dopamine cells placed into the striatum with viral over-expression of alpha synuclein

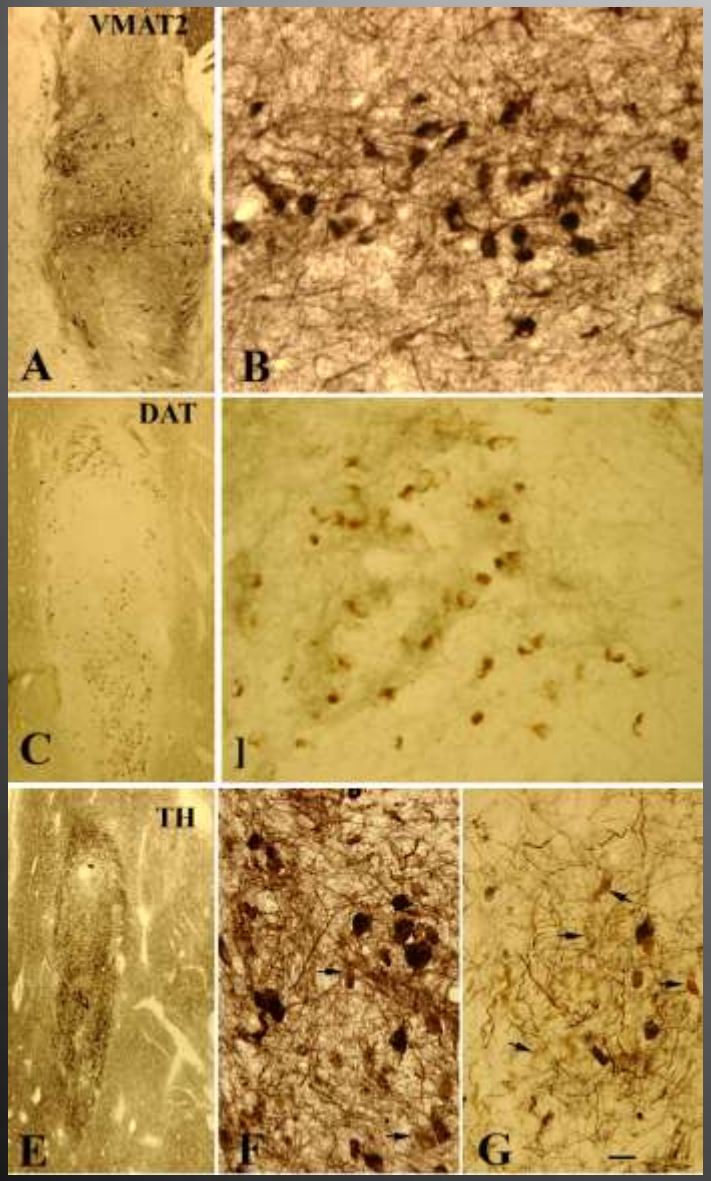
Note the physical segregation of the graft (brown) and gene delivery (black)



A small percentage (5%) of grafted neurons retrogradely transported host-derived alpha synuclein




% of Ubiquitin-ir nigral neurons
0
1
2
3
4
5
6
7
8
9
8.1
6
Graft
Host



Case 2

PD changes in grafted neurons occur that Are analogous to what is seen within nigral neurons in PD

DBS

RESEARCH ARTICLE

Beyond Nine Years of Continuous Subthalamic Nucleus Deep Brain Stimulation in Parkinson's Disease

Maurizio Zibetti, MD, PhD, Aristide Merola, MD, Laura Rizzi, PhD, Valeria Ricchi, MD, Serena Angrisano, MD, Corrado Azzaro, MD, Carlo Alberto Artusi, Nichy Arduino, Alice Marchisio, Michele Lanotte, MD, Mario Rizzone, MD, and Leonardo Lopiano, MD, PhD

Department of Neuroscience, University of Torino, Torino, Italy

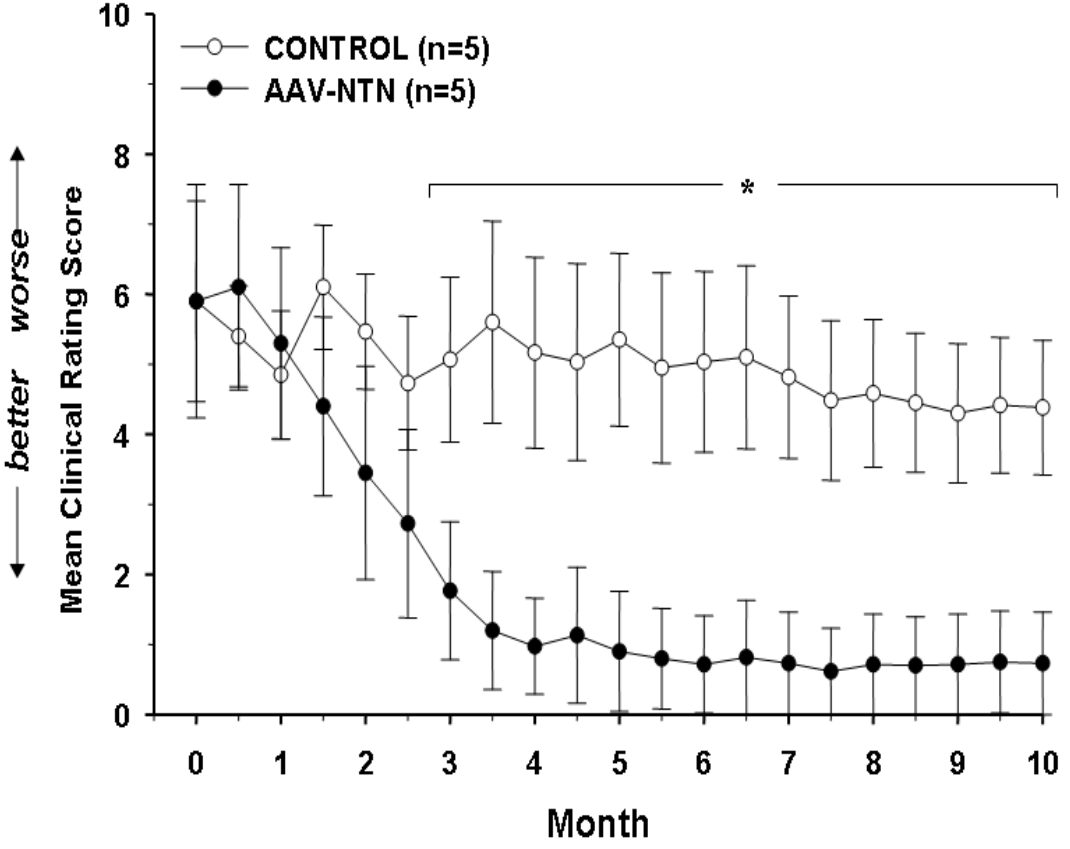
ABSTRACT: Deep brain stimulation of the subthalamic nucleus is an effective treatment for advanced Parkinson's disease. The benefits of bilateral subthalamic stimulation are well documented, and some studies reported outcomes with a follow-up of 5 to 6 years; nevertheless, few data are available beyond 5 years. We report a long-term prospective evaluation of 14 consecutive parkinsonian patients, treated by bilateral subthalamic stimulation for at least 9 years. Motor symptoms, activity of daily living, and motor complications were evaluated by means of the Unified Parkinson's Disease Rating Scale, while cognition and mood were assessed with a specific neuropsychological test battery; medication intake, stimulation parameters, comorbidity, and adverse events were also recorded. Patients were evaluated before surgery and at 1, 5, and ≥9 years after surgery. At last follow-up, deep brain stimulation significantly improved the motor score by 42% compared to baseline, whereas activities of daily living were no longer improved; there was a 39% reduction in the dosage of dopaminergic drugs and a 59% improvement of L-dopa-related motor complications. The neuropsychological assessment showed that 4 patients (29%) developed a significant cognitive decline over the follow-up period. These results indicate a persistent effect of deep brain stimulation of the subthalamic nucleus on the cardinal motor symptoms in advanced Parkinson's disease patients in the long-term; however, a worsening of patients' disability, mainly due to disease progression, was observed. © 2011 *Movement* Disorder Society

Key Words: Parkinson's disease; deep brain stimulation; subthalamic nucleus; long-term follow-up

Do We Need Cell Replacement?

- The major unmet needs in PD are not levodopa-responsive motor deficits but levodopa non-responsive motor deficits (e.g. gait disturbance) and non-motor PD (e.g. depression, dementia, constipation, sleep disturbance). There is no reason to believe these symptoms would benefit from DA cell replacement.
- The patient population that would benefit from DA cell replacement is the same one that would benefit from DBS.
- The symptoms that would benefit from cell replacement are the same ones that would benefit from DBS (levodopa responsive symptoms).
- There is no evidence that the graft would be neuroprotective

AAV2-Neurturin (Cere-120) reverses parkinsonian signs in MPTP-Treated monkeys



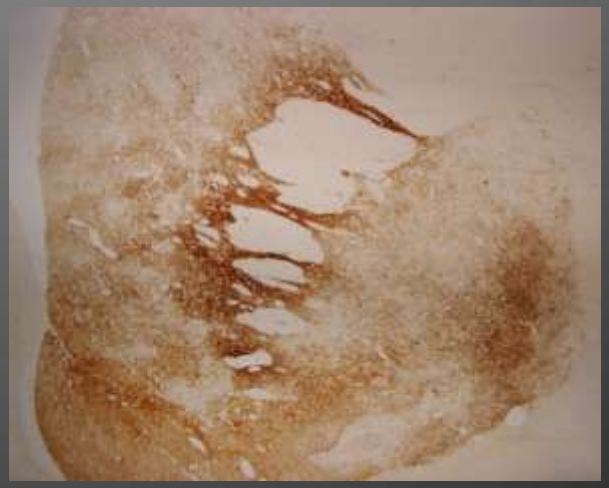
*p<0.01-0.05

Preservation of striatal dopamine by AAV2-Neurturin

Control Side

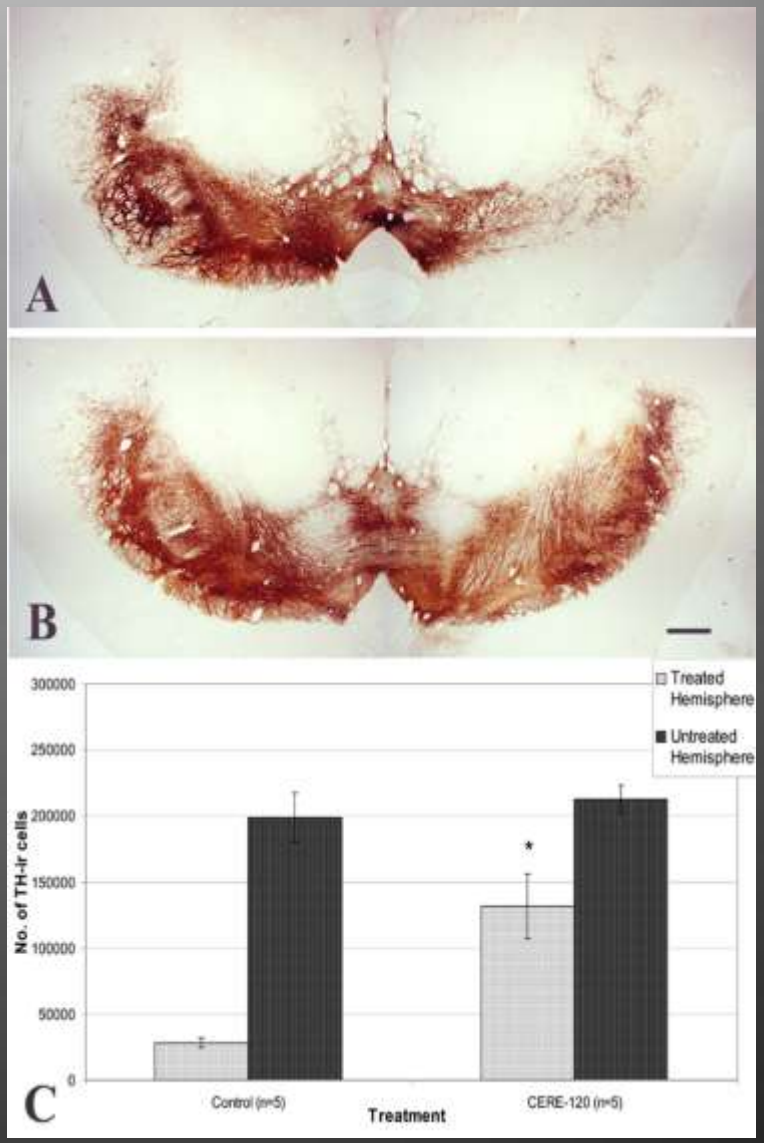
AAV-GFP

Control Side

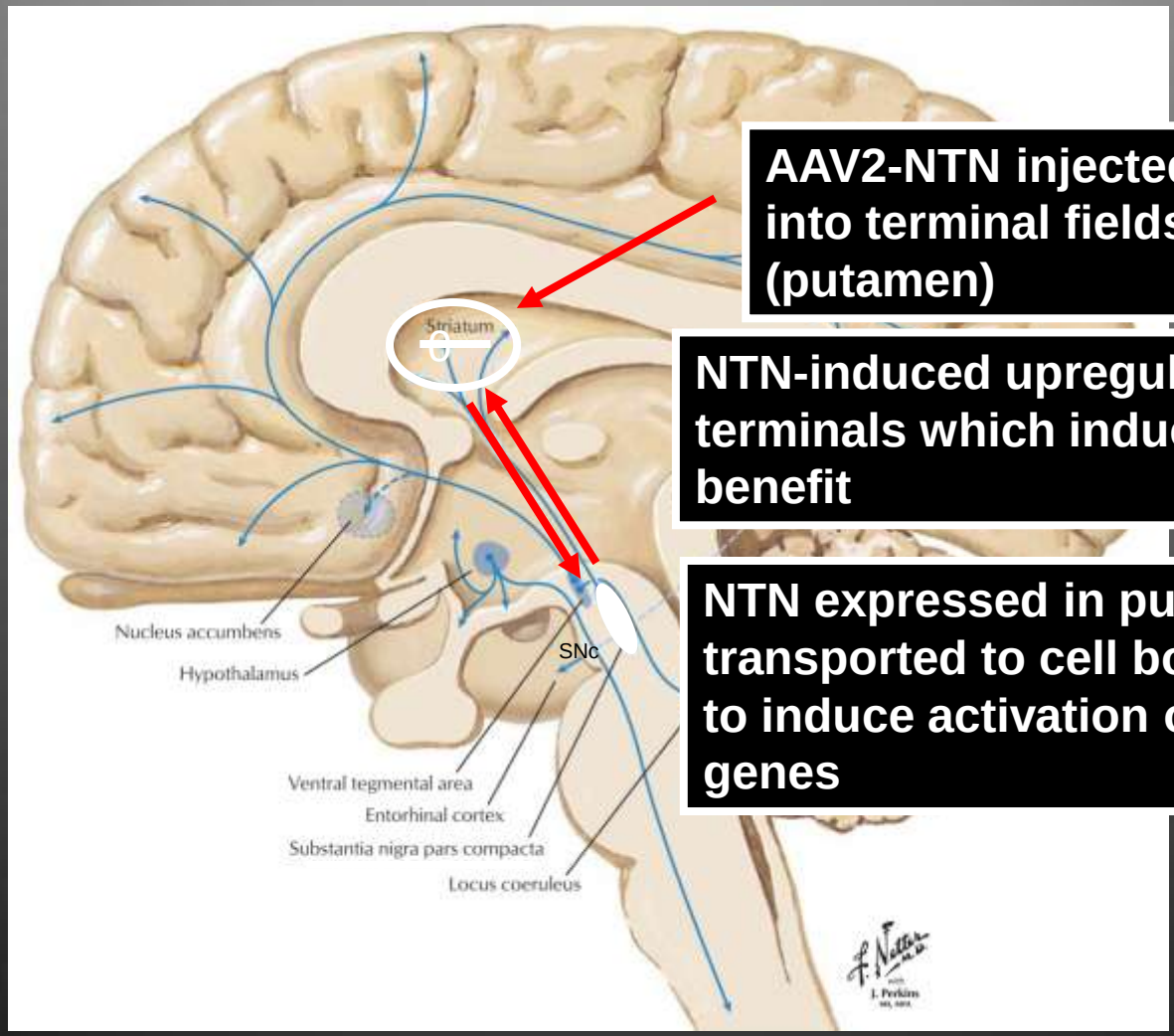


AAV-NTN

Preservation of TH-ir nigral perikarya by AAV2-neurturin



Targeting Nigrostriatal Neurons With AAV-2 NTN

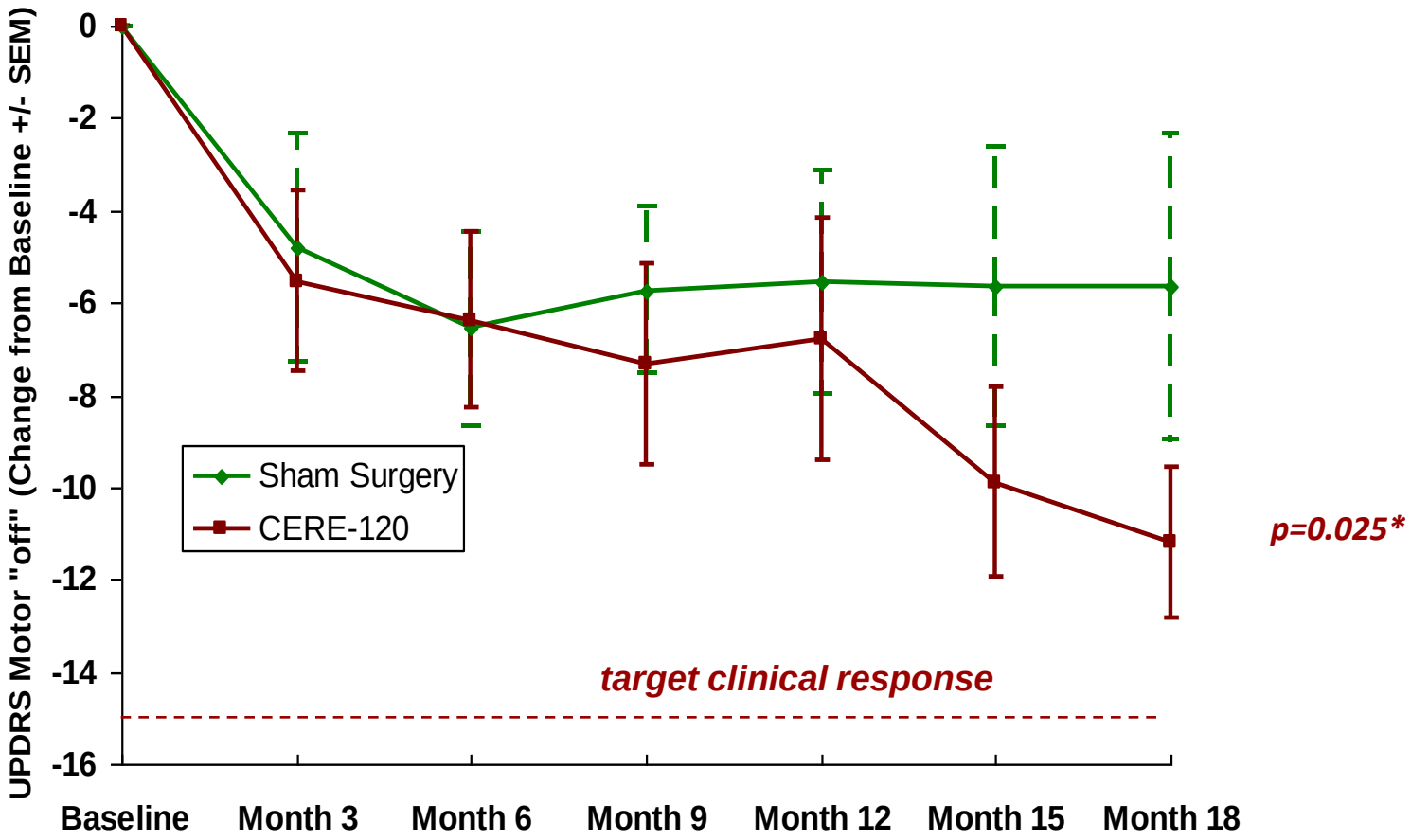


AAV2-NTN injected into terminal fields (putamen)

NTN-induced upregulation of DA terminals which induce clinical benefit

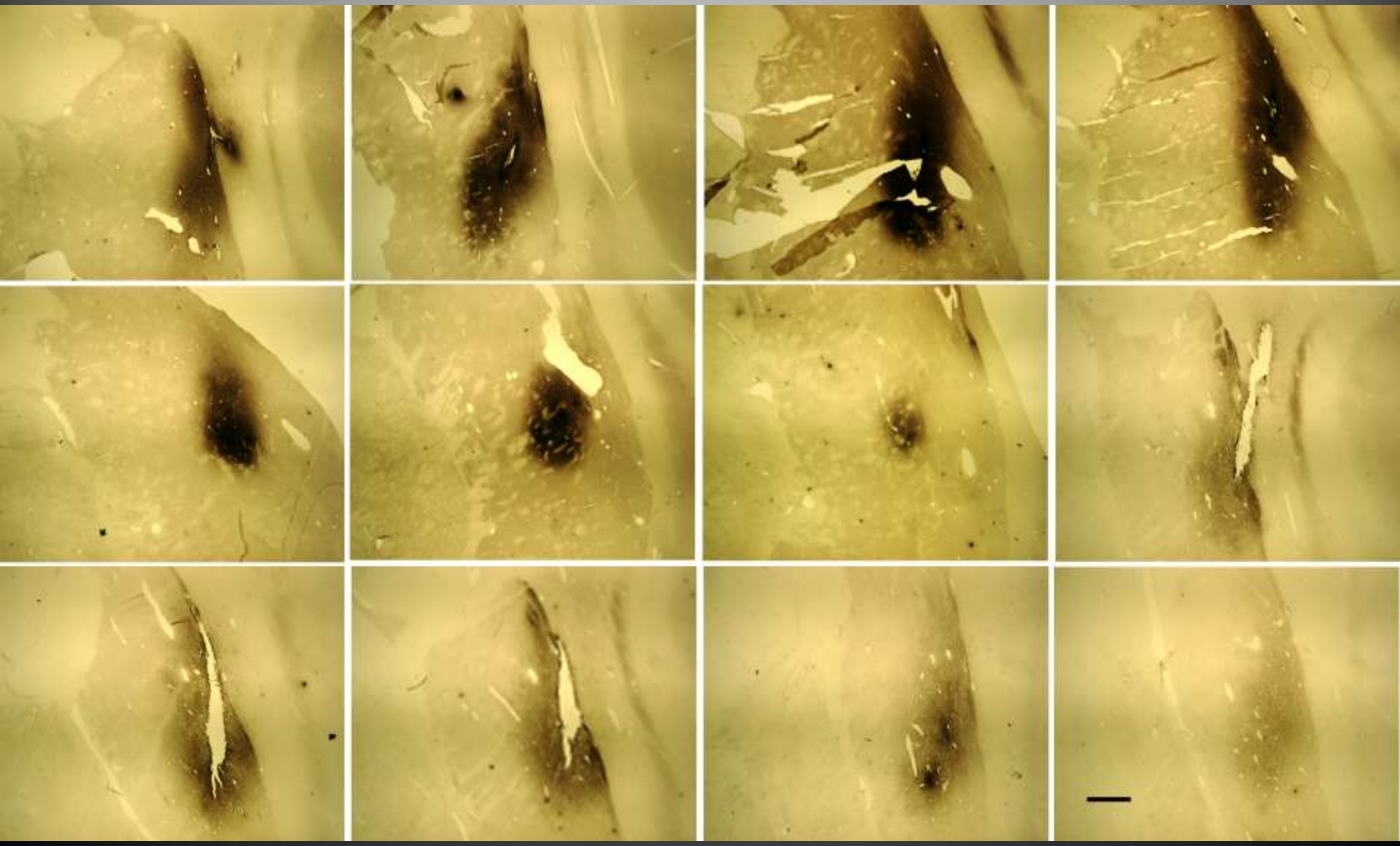
NTN expressed in putamen and transported to cell bodies in nigra to induce activation of repair genes

Change From Baseline in UPDRS (Part III) Motor Score "off" (Blinded data; N=30)

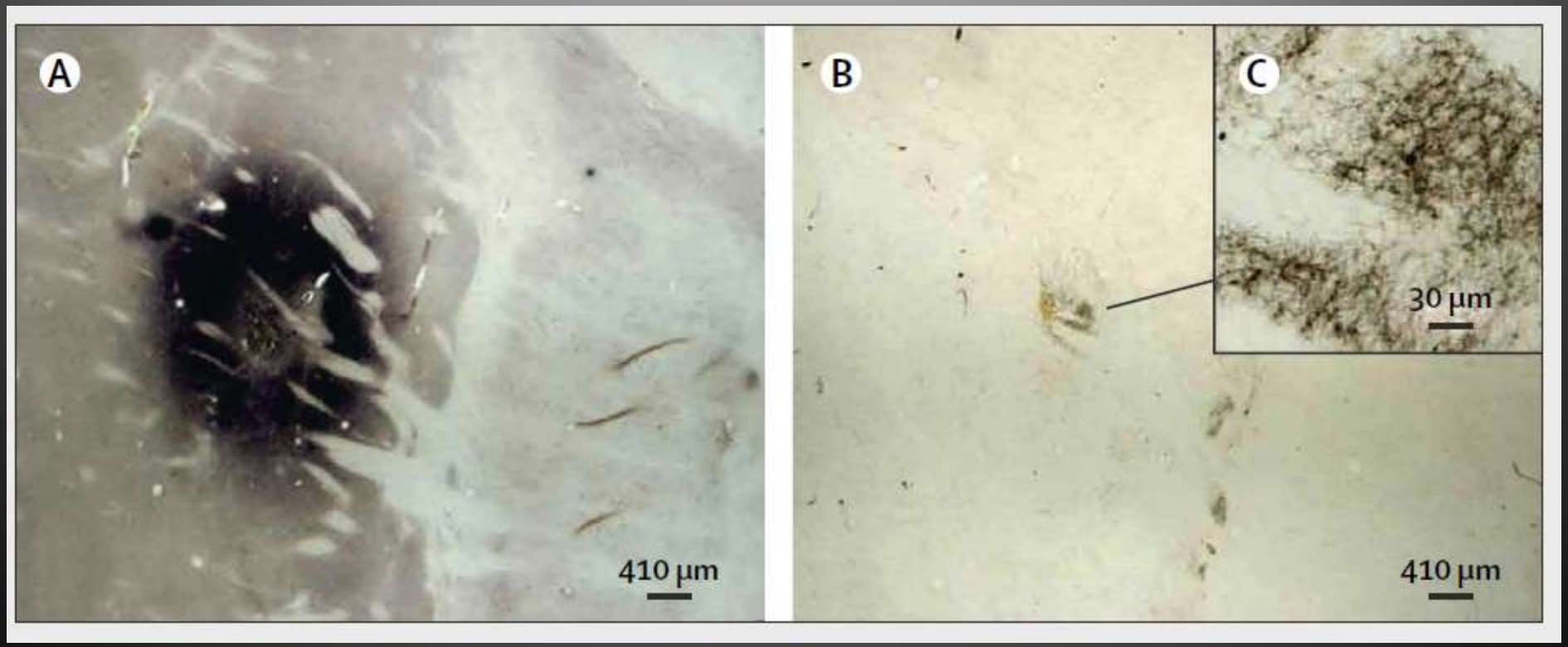


** ANCOVA model with a main effect for treatment group and baseline UPDRS Part III motor score in the practically defined off condition as covariate. Note: at 18 mos, 14 subjects have scores; therefore 16 subjects: LOCF*

Cere-120-2-Right



NTN Staining in Putamen of PD Patient Following AAV2-NTN Gene Delivery



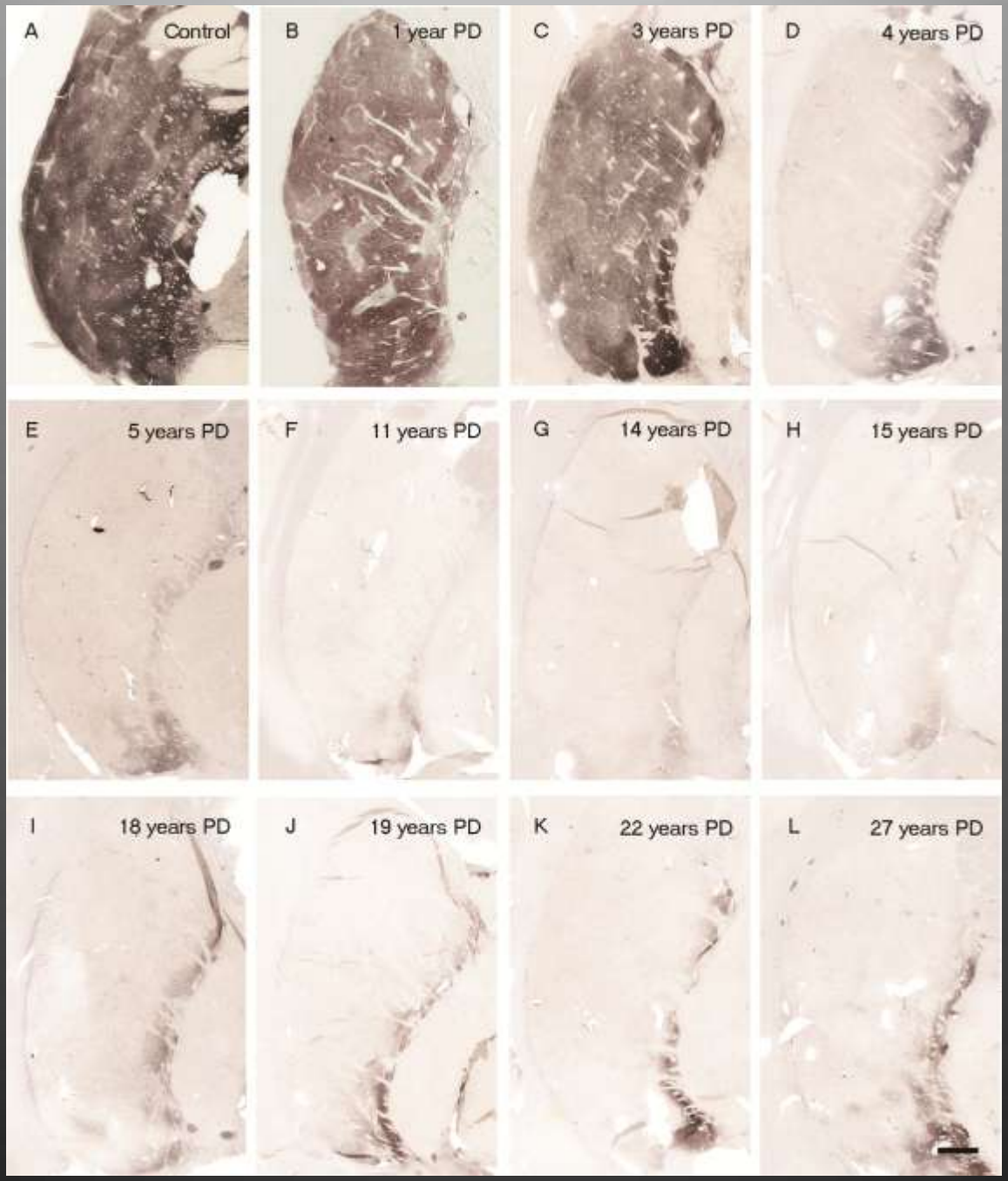
Bartus et al, Mov Disord 2011

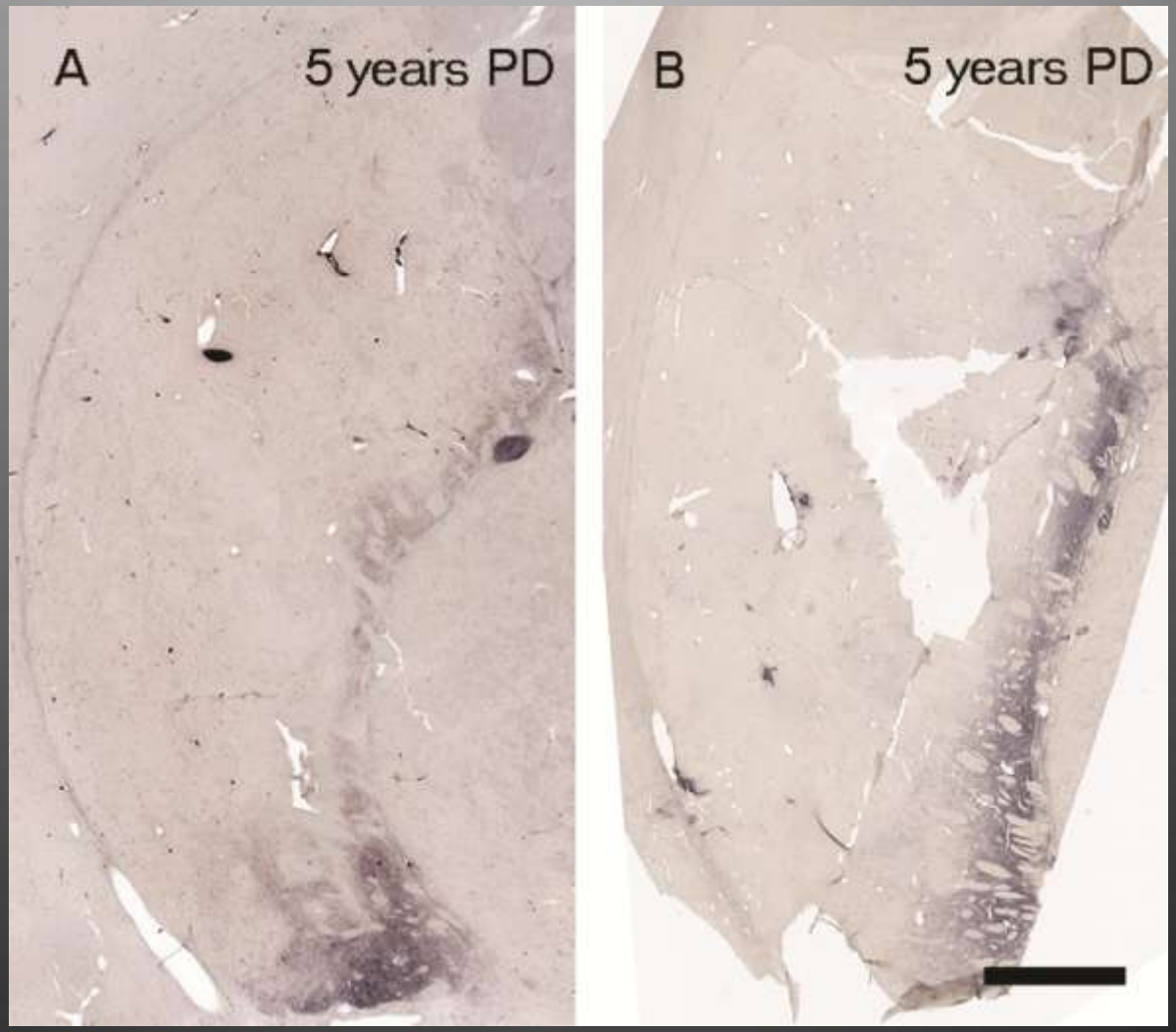
Table 1

Cases	Arizona cohort	Age (years)	PMI (hours)	Disease durations
1	98-15	88	NA	1
2	96-36*	88	NA	3
3	03-45	88	1.83	4
4	95-19*	84	NA	4
5	99-08	64	3.75	5
6	93-19*	80	NA	5
7	04-01^	89	6.50	7
8	98-38*	64	1.00	11
9	99-26	79	6.00	11
10	07-40	69	4.16	11
11	01-39^	85	2.00	13
12	04-10^	77	1.66	14
13	04-27	79	3.50	14
14	01-42	85	4.00	15
15	07-01*^	63	18.50	15
16	03-20^	81	4.00	18
17	05-26	73	7.16	18
18	06-62	82	4.16	19
19	99-17^	83	12.00	21
20	02-18	74	4.00	21
21	02-17	82	3.00	22
22	98-03	72	3.00	27

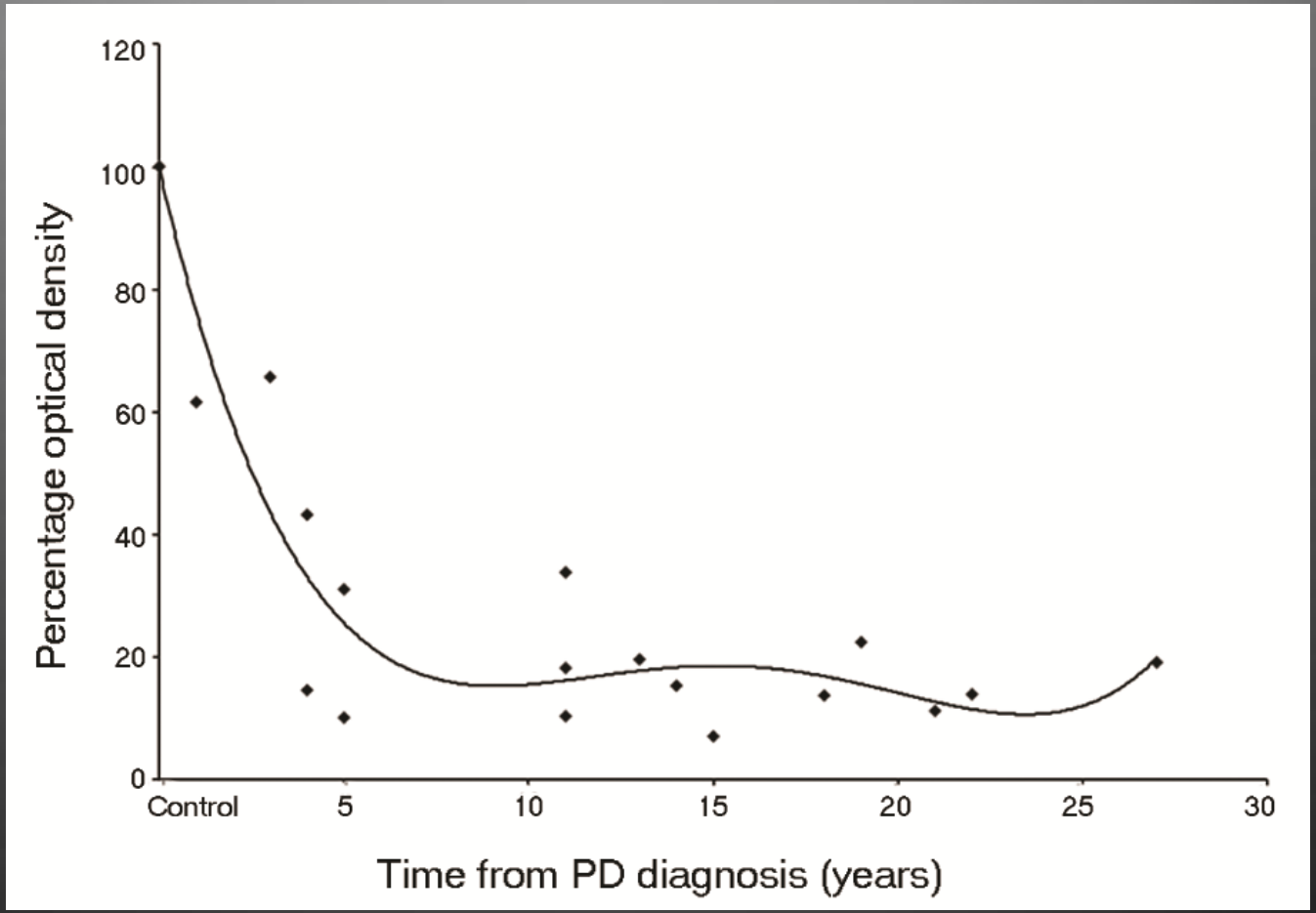
Cases	Australia cohort	Age (years)	PMI (hours)	Disease durations
1	Case#1	67	8.00	3
2	Case#2	86	13.00	3
3	Case#3	80	12.00	3
4	Case#4	84	4.50	4
5	Case#5	43	36.00	4
6	Case#6	53	5.00	5

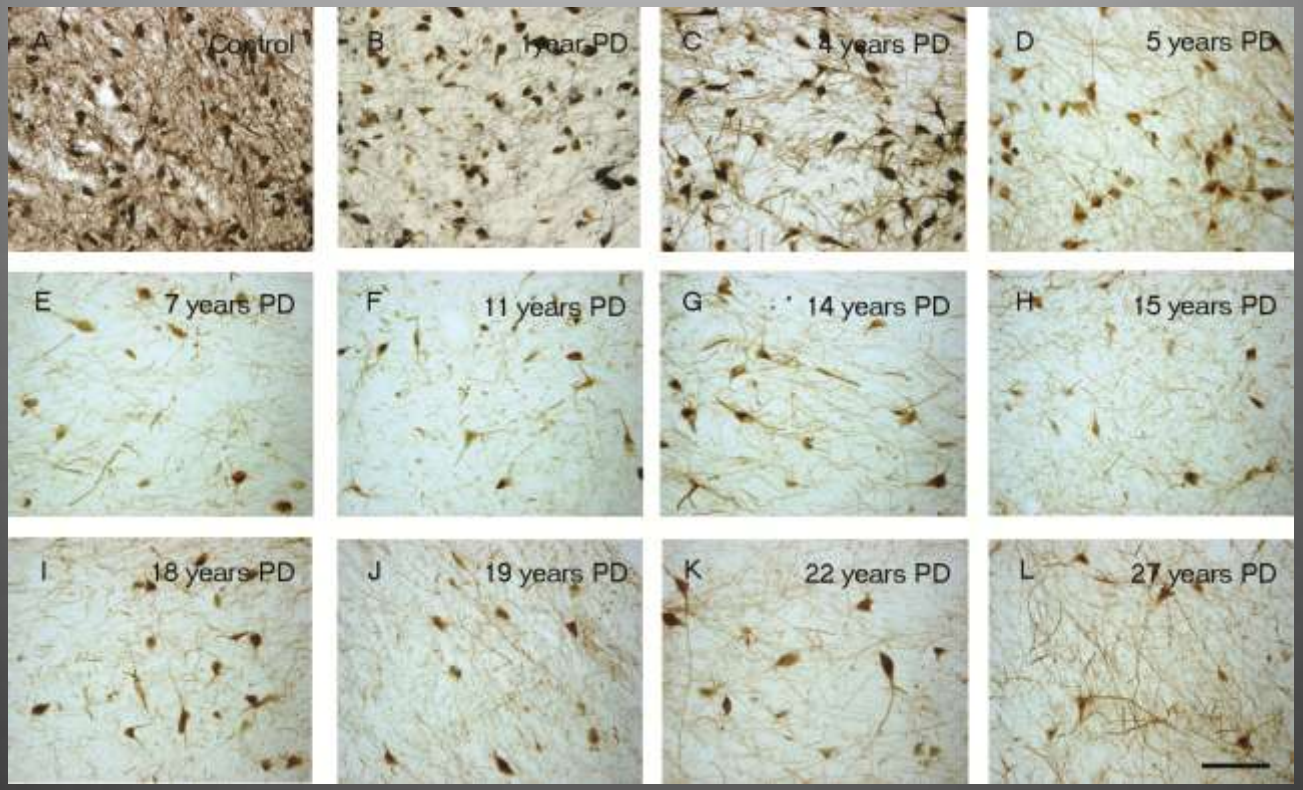
Cases	Controls	Age (years)	PMI (hours)	
1	B98-57	91	4.00	
2	B98-115	81	4.00	
3	B97-39	83	5.50	
4	B98-54	91	10.70	
5	B00-76	71	4.10	
6	B96-50	88	7.00	
7	B96-98	72	7.10	

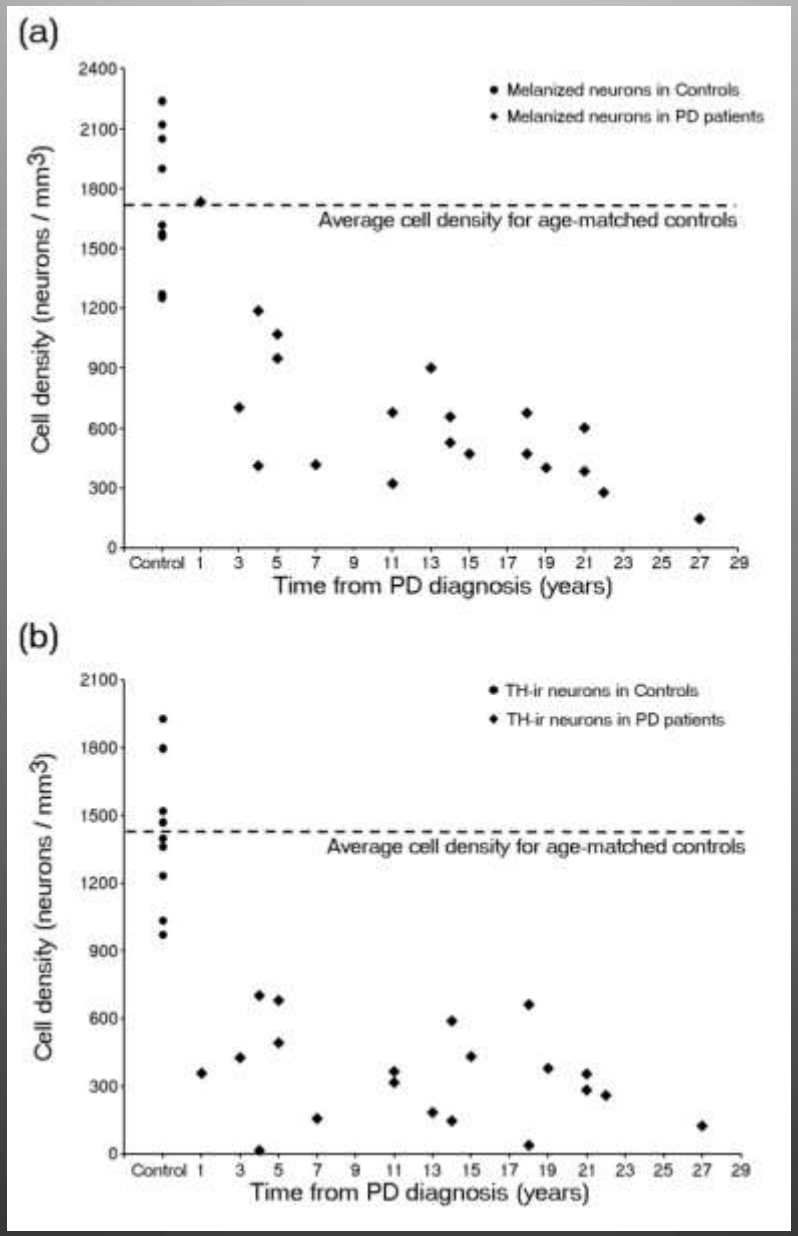

A
Control
B
1 year PD
C
3 years PD
D
4 years PD
E
5 years PD
F
11 years PD
G
14 years PD
H
15 years PD
I
18 years PD
J
19 years PD
K
22 years PD
L
27 years PD


A
5 years PD
B
5 years PD

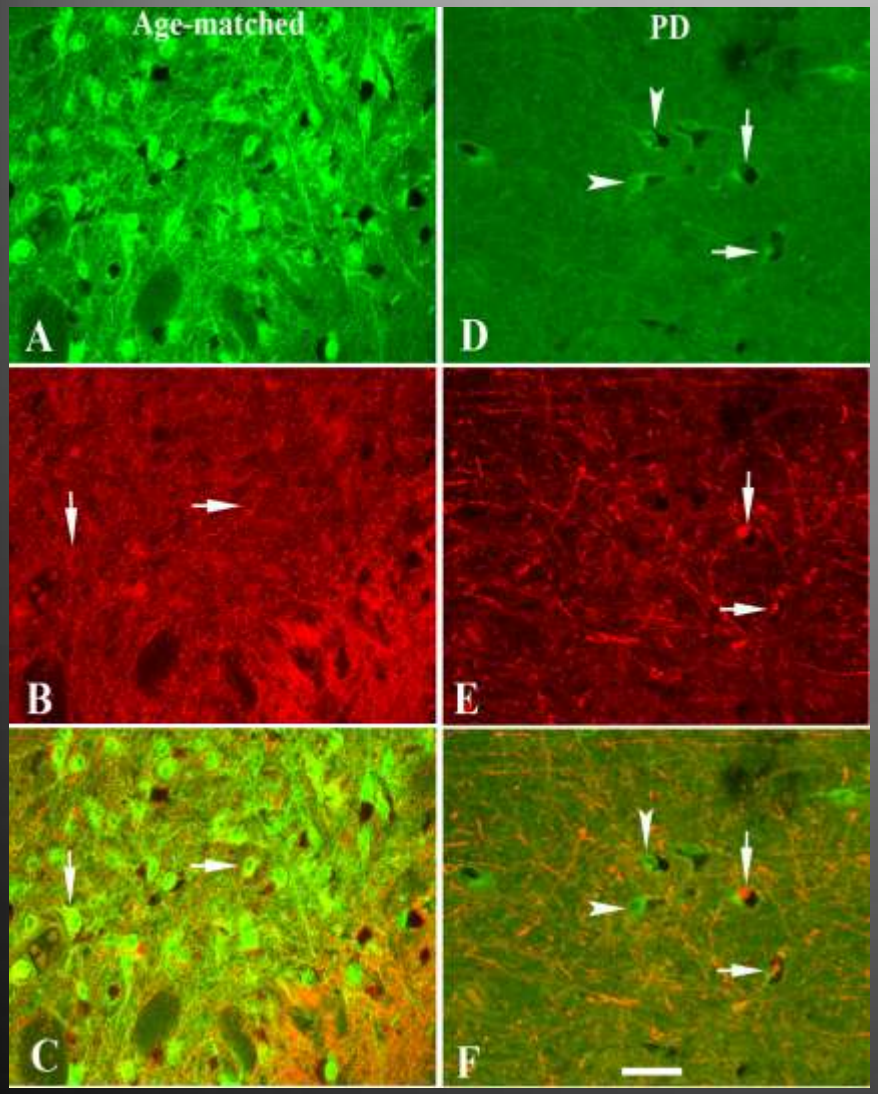
Optical density of TH-ir putamenal neurons as a function of disease duration

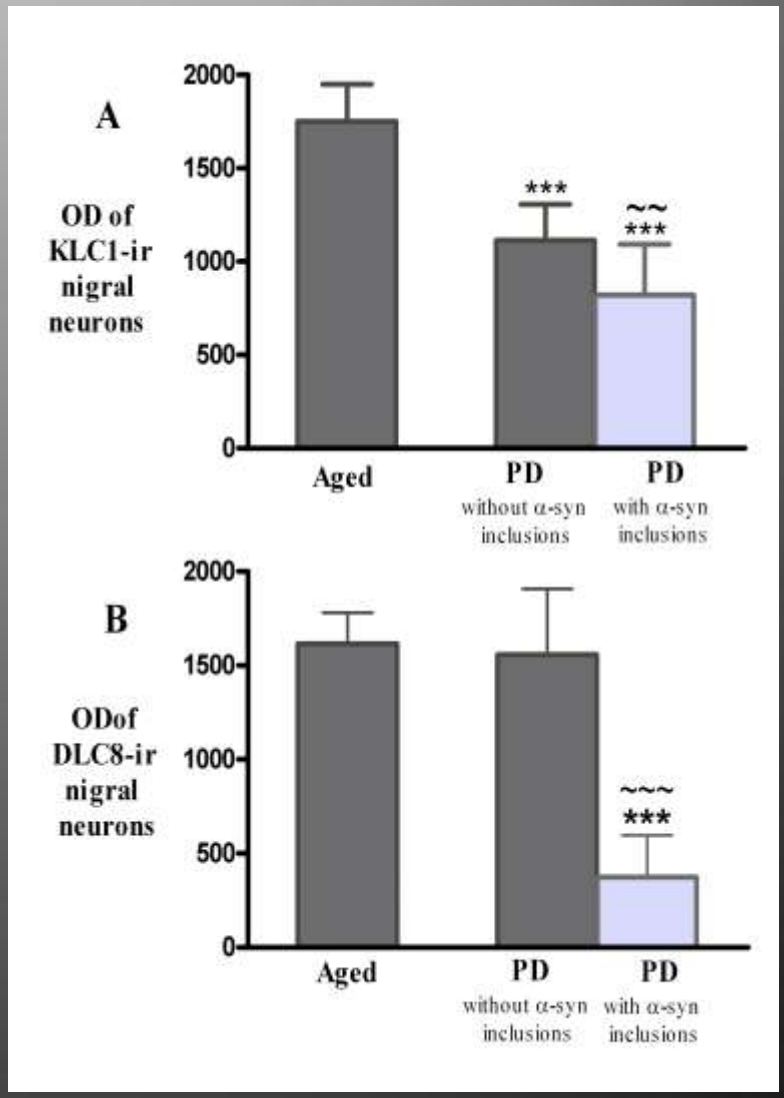



A Control
B 1 year PD
C 4 years PD
D 5 years PD
E 7 years PD
F 11 years PD
G 14 years PD
H 15 years PD
I 18 years PD
J 19 years PD
K 22 years PD
L 27 years PD


(a)
Melanized neurons in Controls
Melanized neurons in PD patients
Average cell density for age-matched controls
Cell density (neurons / mm3)
2400
2100
1800
1500
1200
900
600
300
0
Control 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Time from PD diagnosis (years)
(b)
TH-ir neurons in Controls
TH-ir neurons in PD patients
Average cell density for age-matched controls
Cell density (neurons / mm3)
2100
1800
1500
1200
900
600
300
0
Control 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Time from PD diagnosis (years)

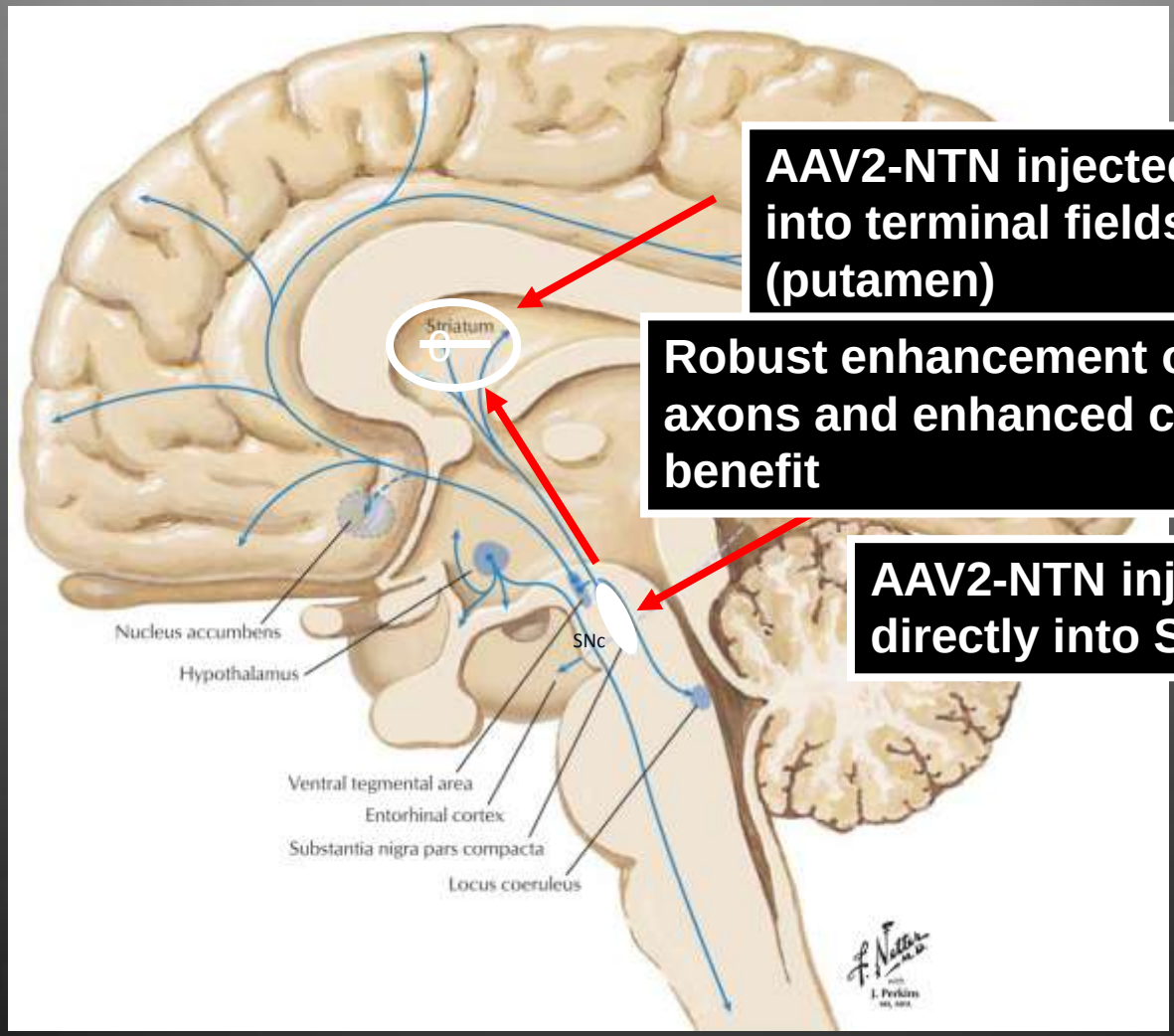
Axonal Transport Defects in PD



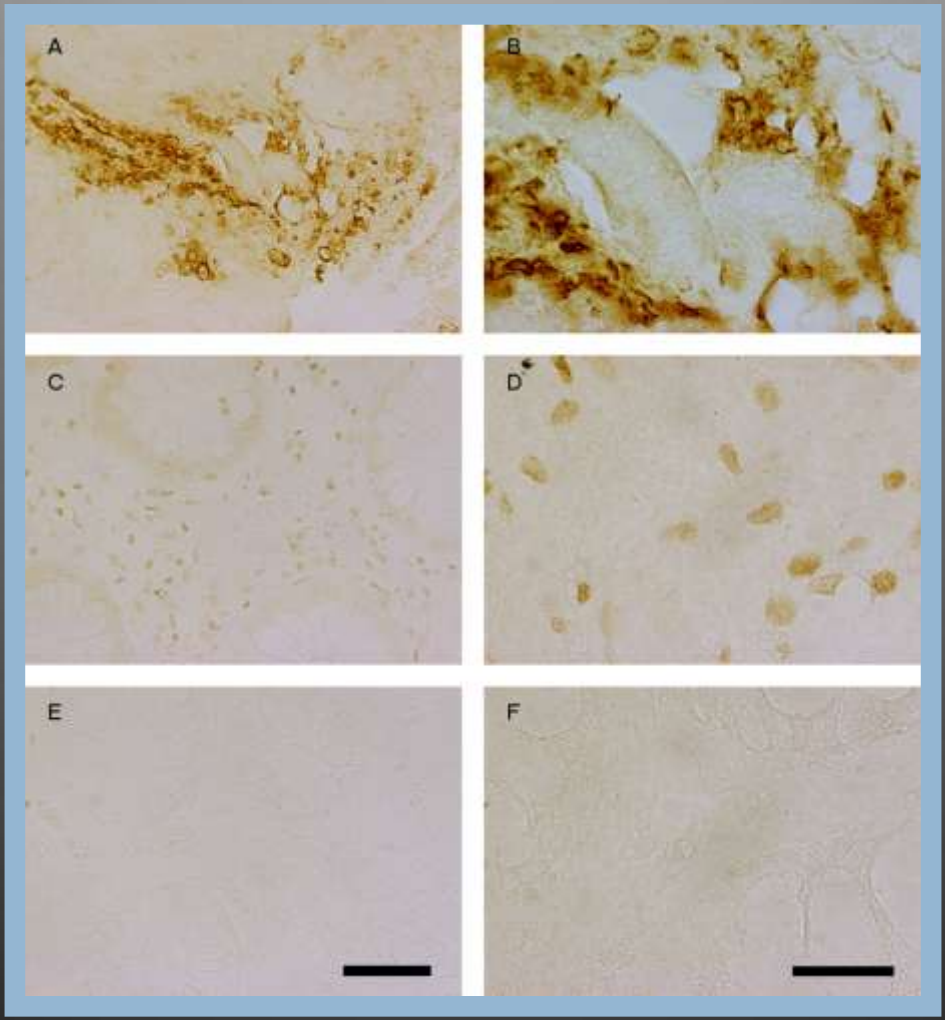


Chu et al., Brain 2012

Targeting Nigrostriatal Neurons With AAV2-NTN in PD



Synuclein staining

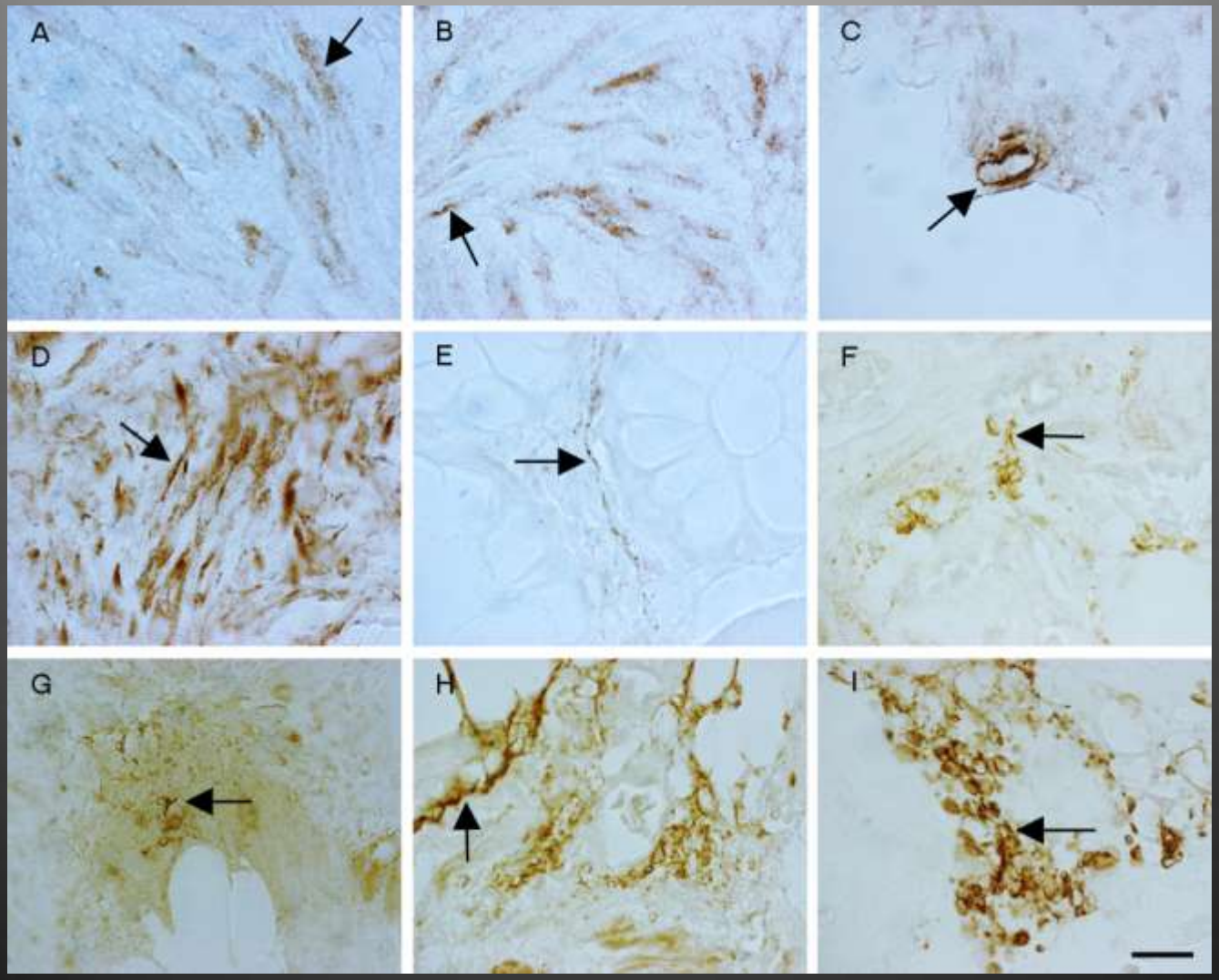


Parkinson disease

Crohn’s disease

Control

Which PD cases get synuclein? All of them!!



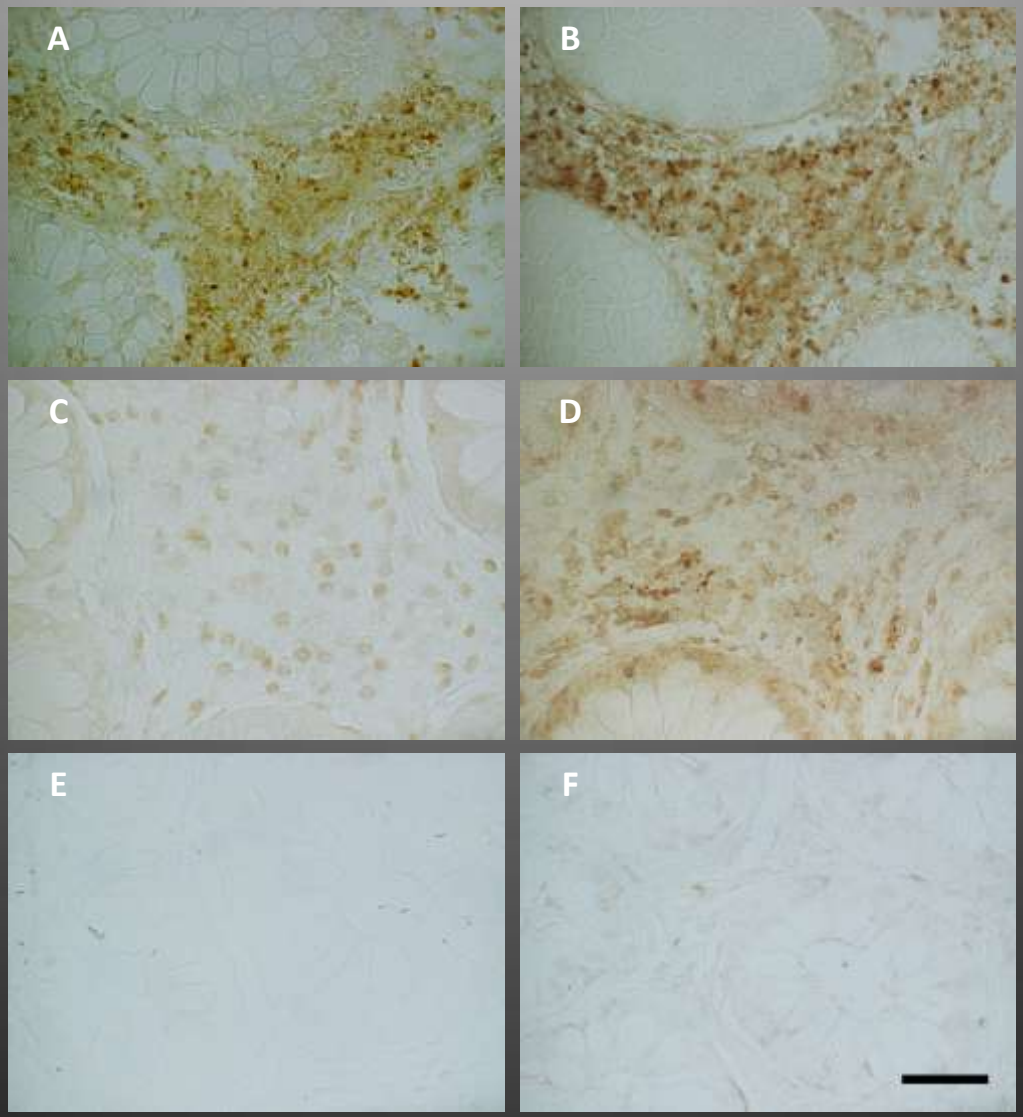
Alpha synuclein

Nitro-tyrosine

Parkinson's

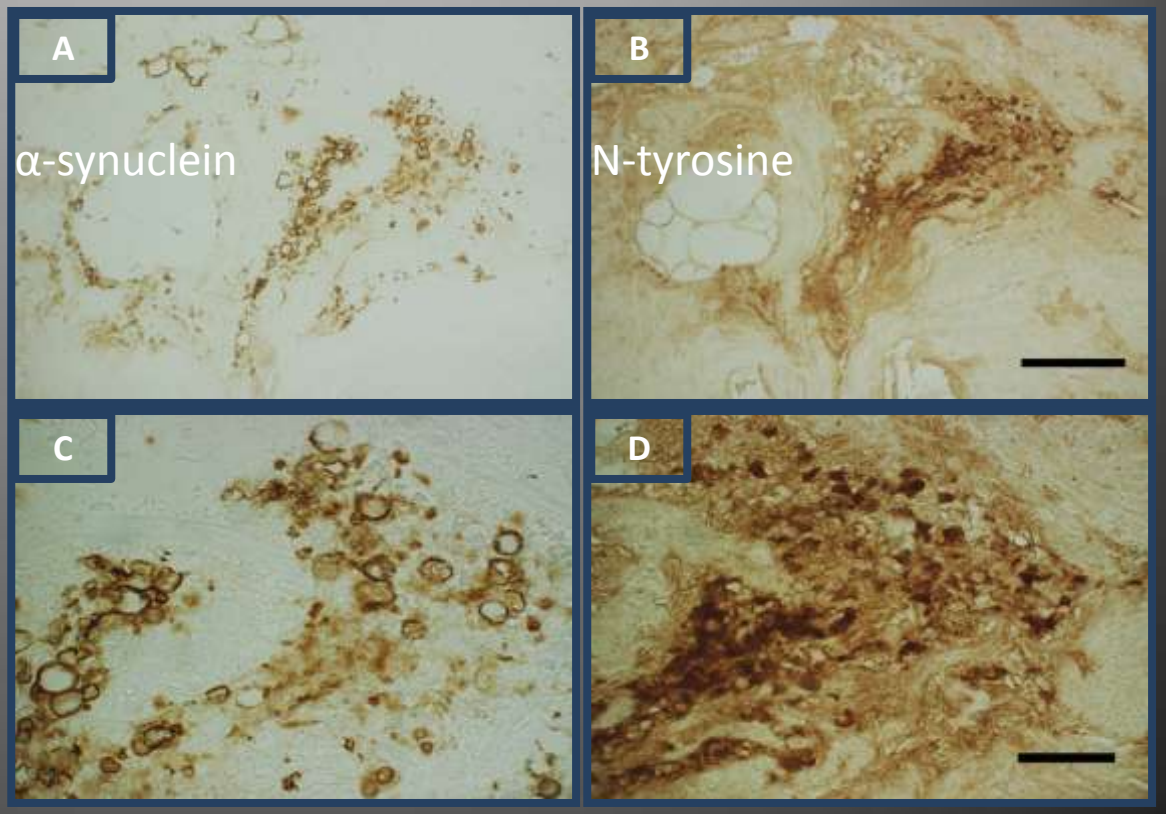
Ulcerative Colitis

Aged-Matched Control



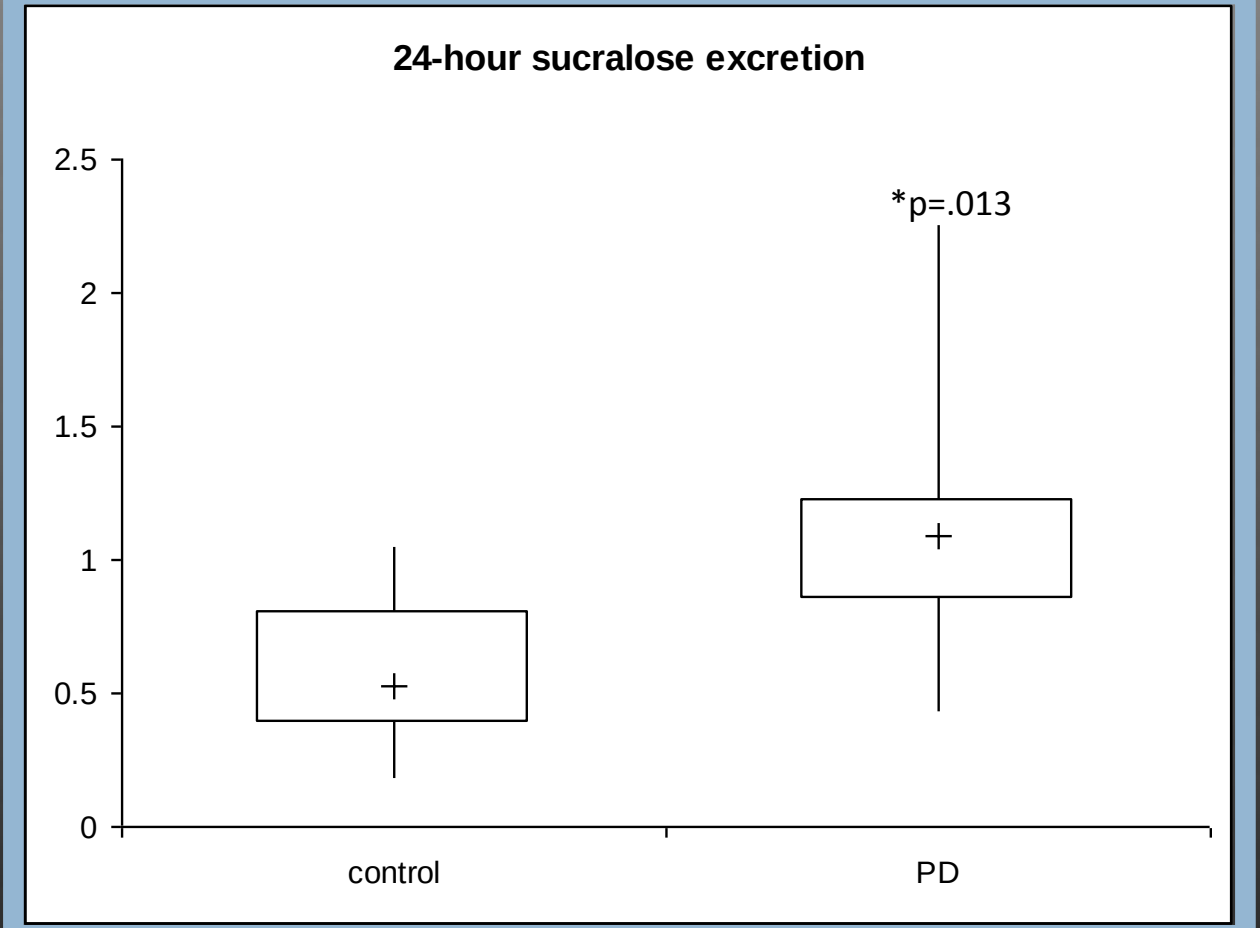
Case study

- 85-y/o woman
- Psychotic depression 2002→ECT
- MCI
- Rest tremor 2/2010
  - Colonic polyp biopsied 2005.

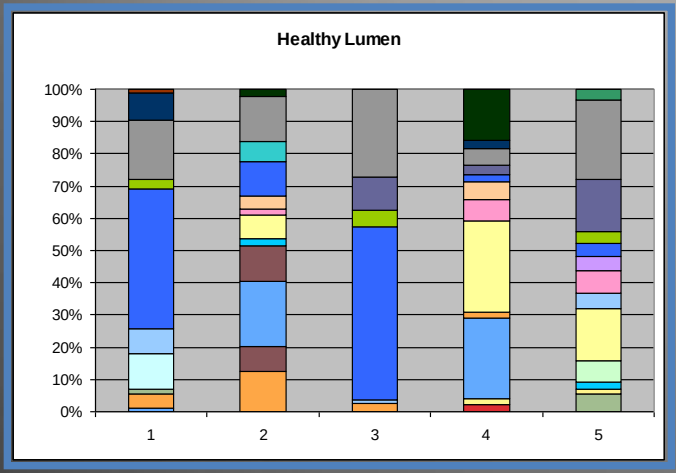


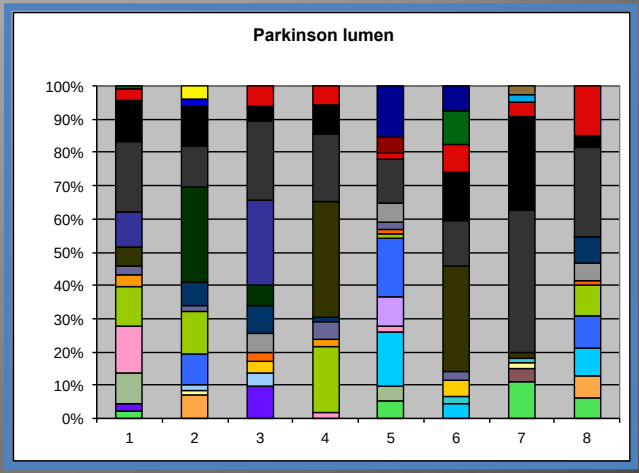
Shannon et al. Movement Disorders ,2012

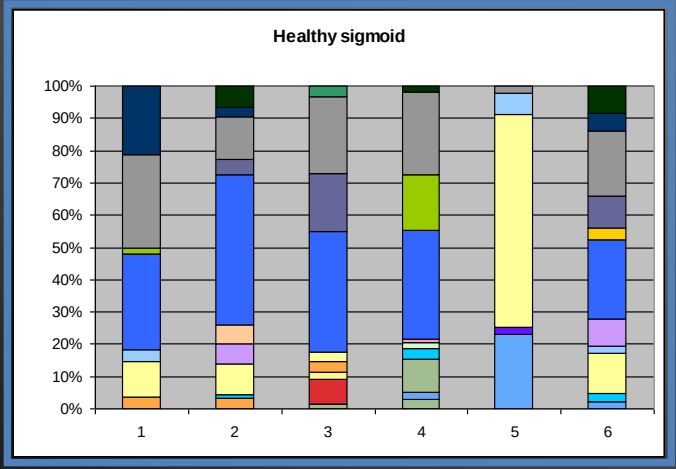
Intestinal permeability in PD

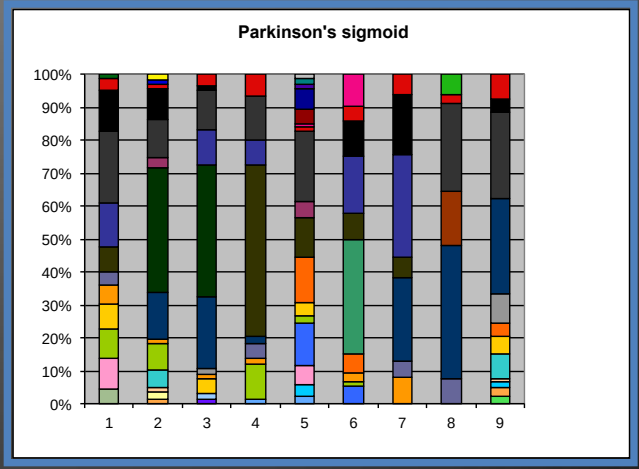


LH-PCR PD gut microbiota

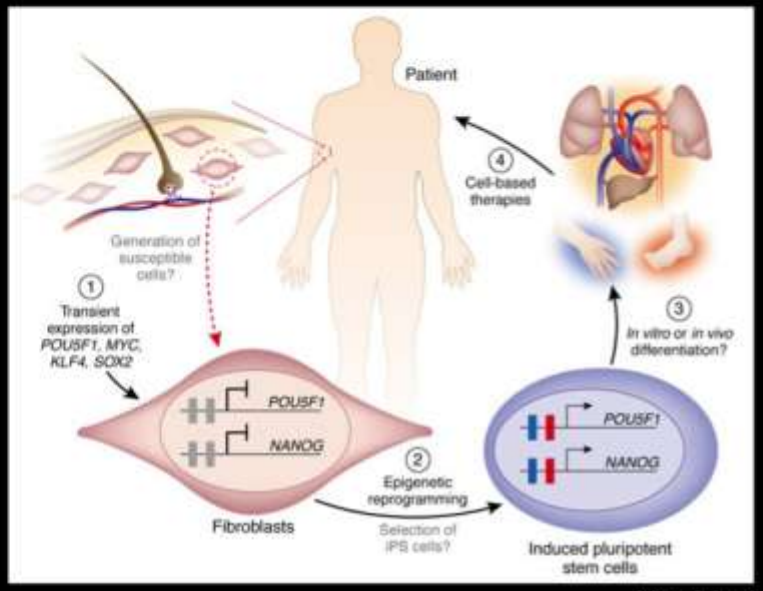




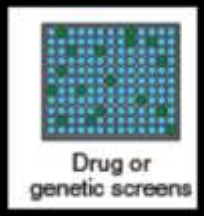




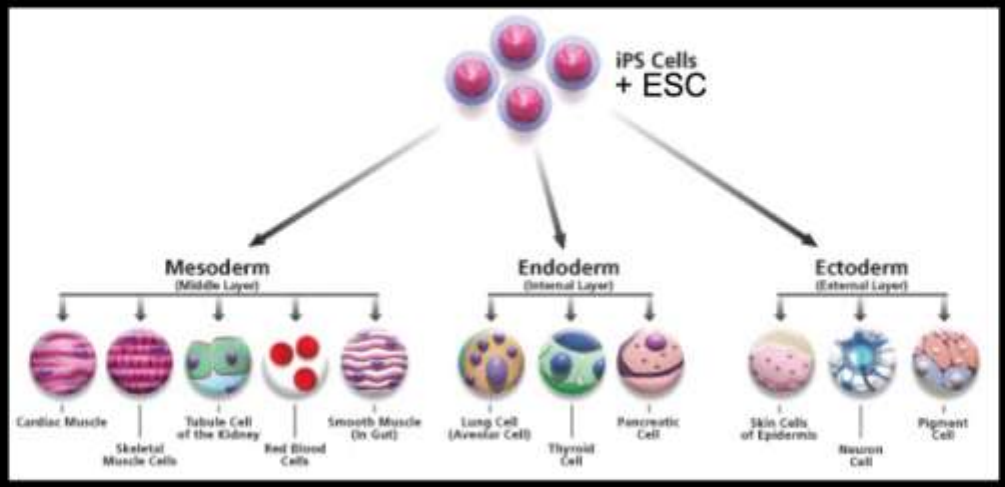
Induced Pluripotent Stem (iPS) Cells



Nat Med

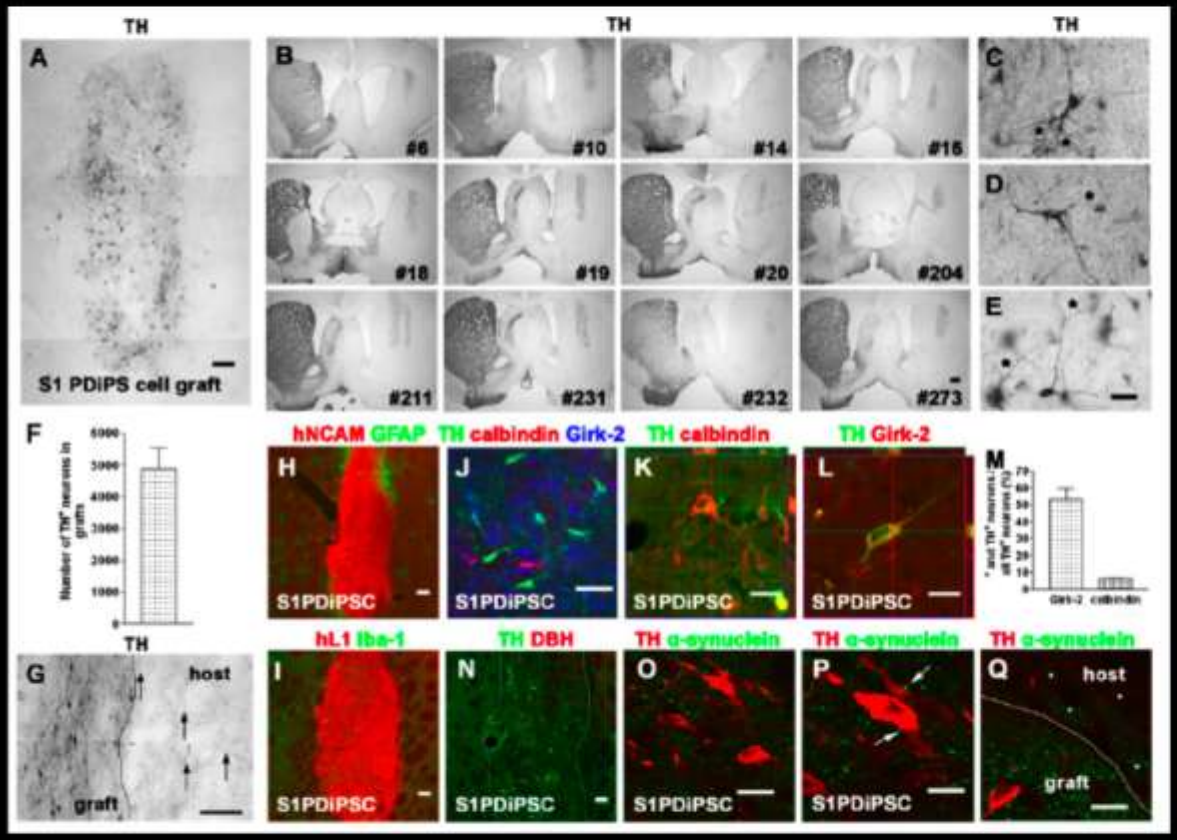


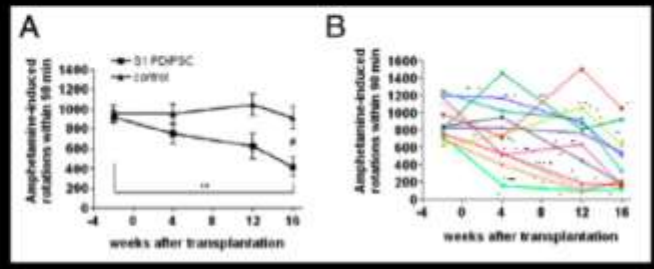
Nat Meth

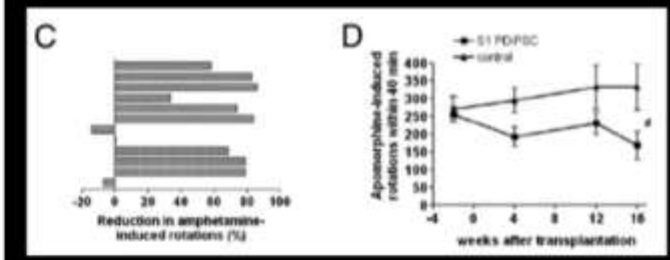


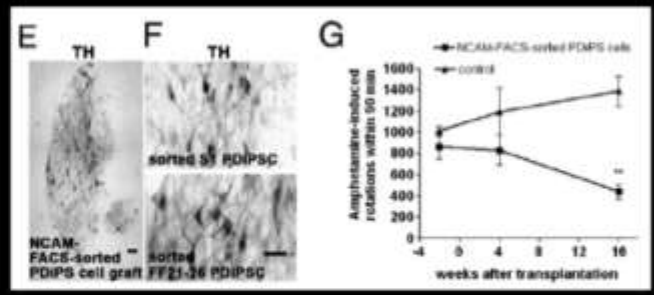
Sigma-Aldrich

PD-Patient Derived iPS-DA Neurons Reduce Motor Asymmetry in PD Rats



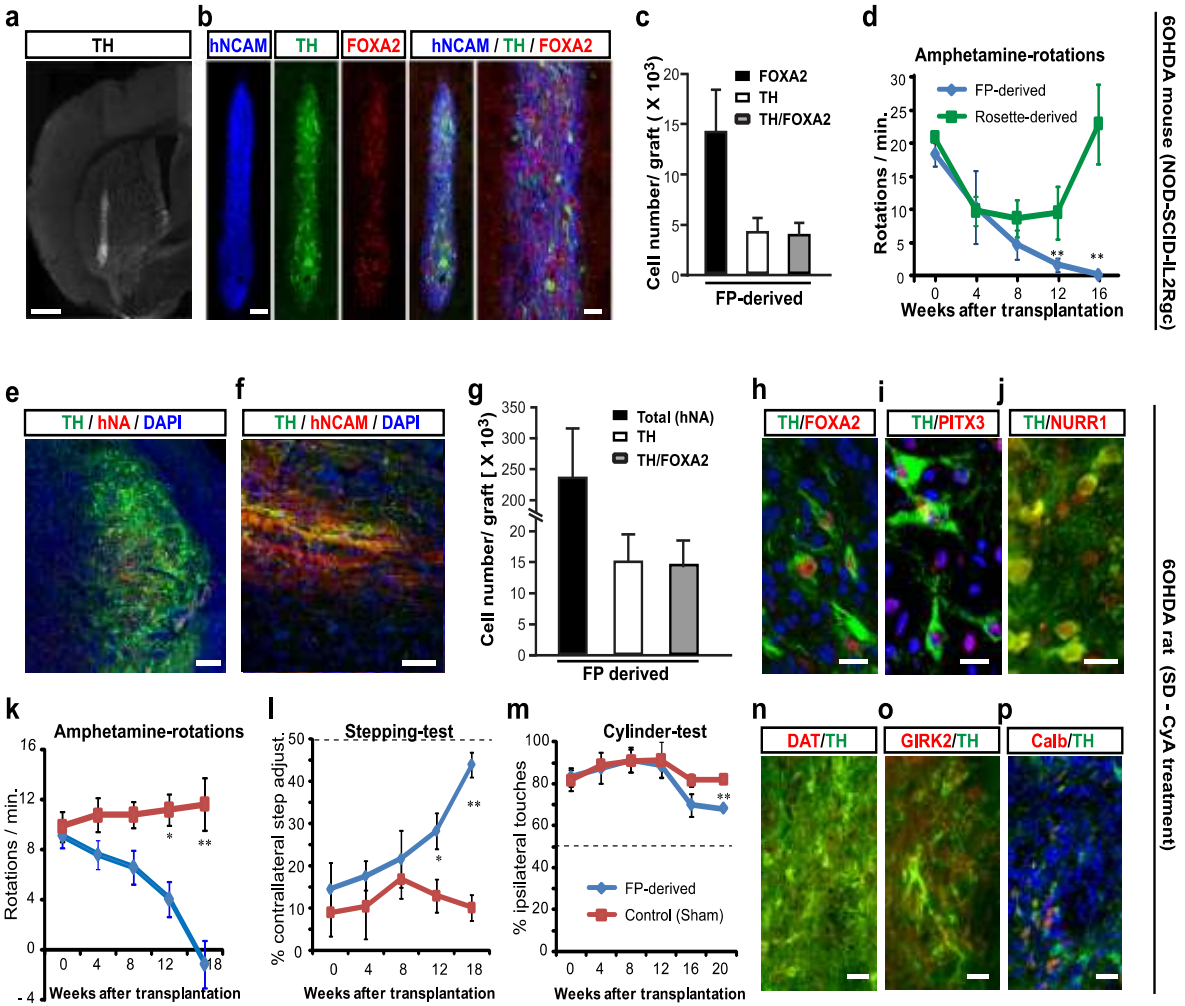


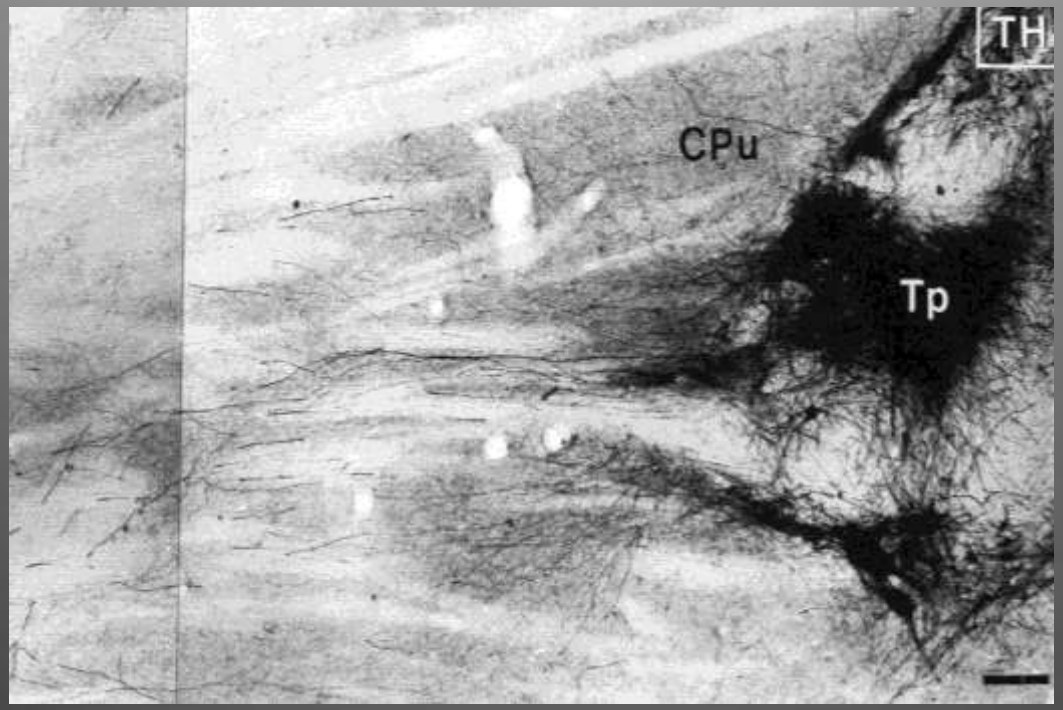




Hargus et al., *PNAS* 2010

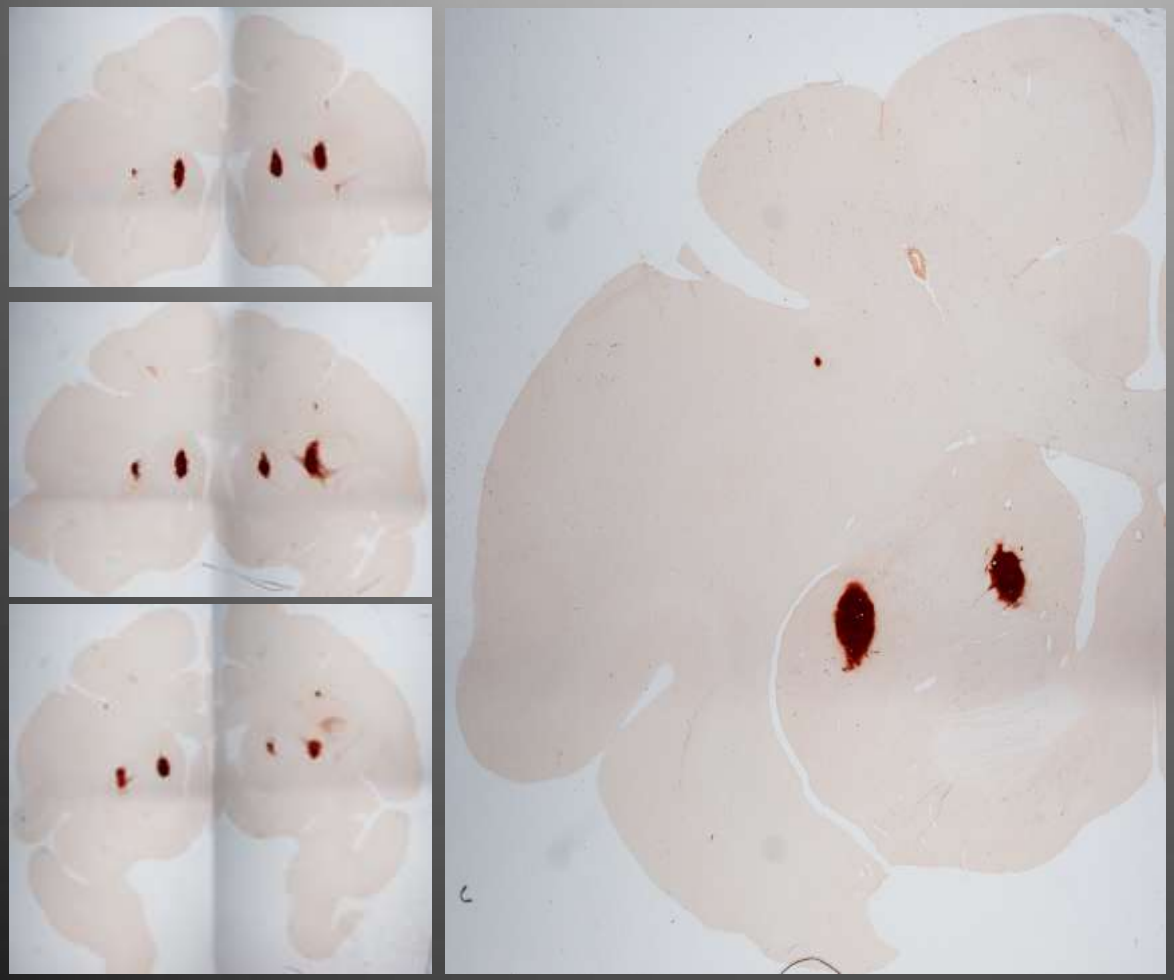
Human Embryonic Stem Cells



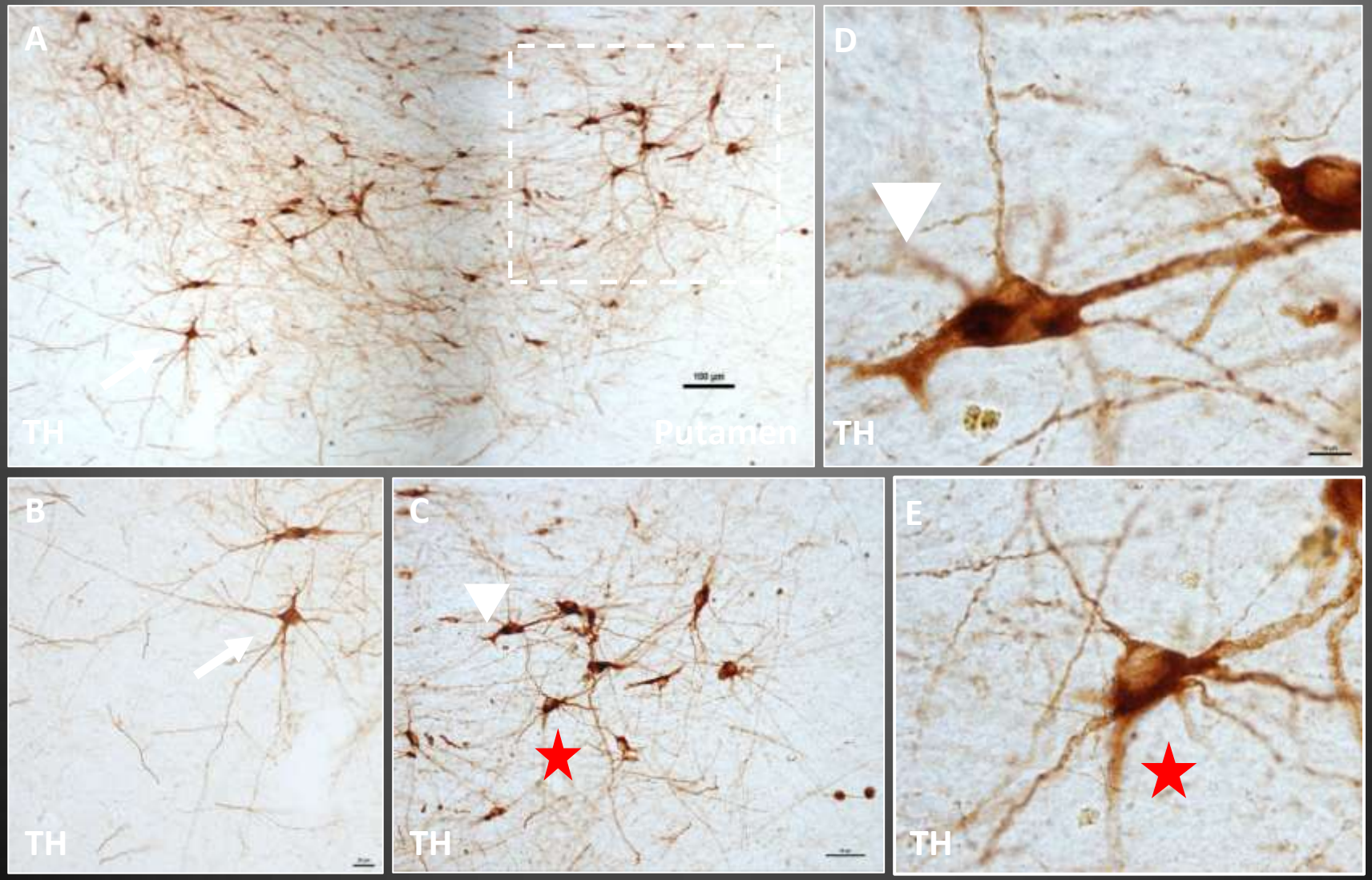


Slide provided by Anders Bjorklund

FP-Derived Human DA Neurons in PD Monkey (3-Month)

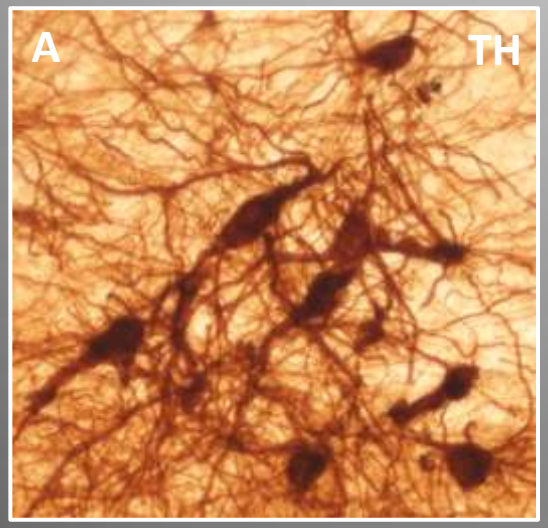


ESC-Derived Human Dopamine Neurons in PD Monkey

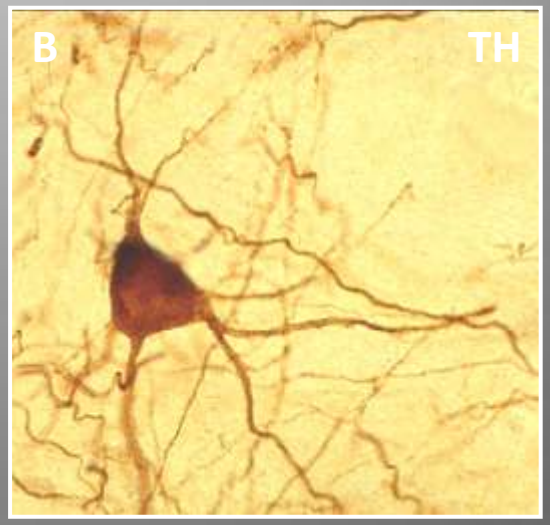


Transplanted HESC-DA cell Retain Midbrain Morphology in MPTP Rhesus Monkey

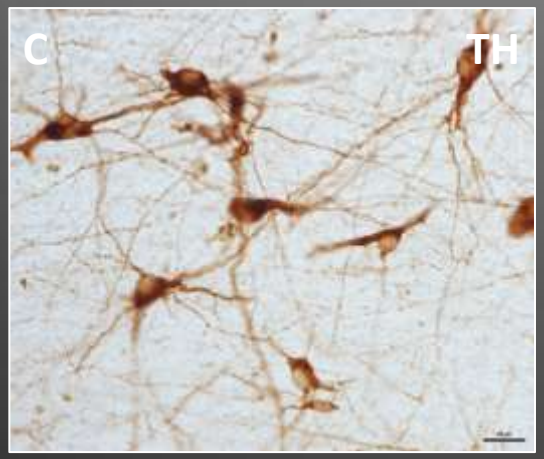
Dopaminergic Phenotype & Cell Morphology



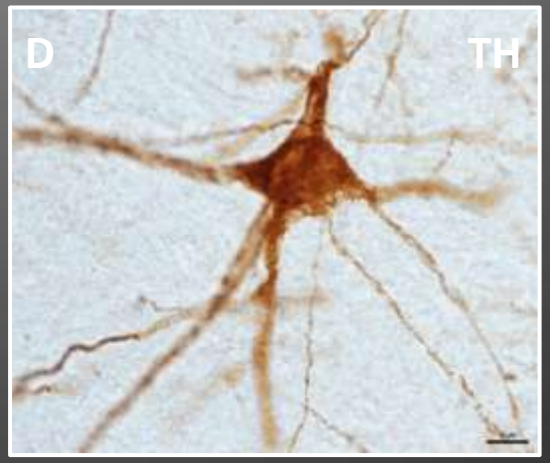
Fetal VM Human PD



Fetal VM Human PD



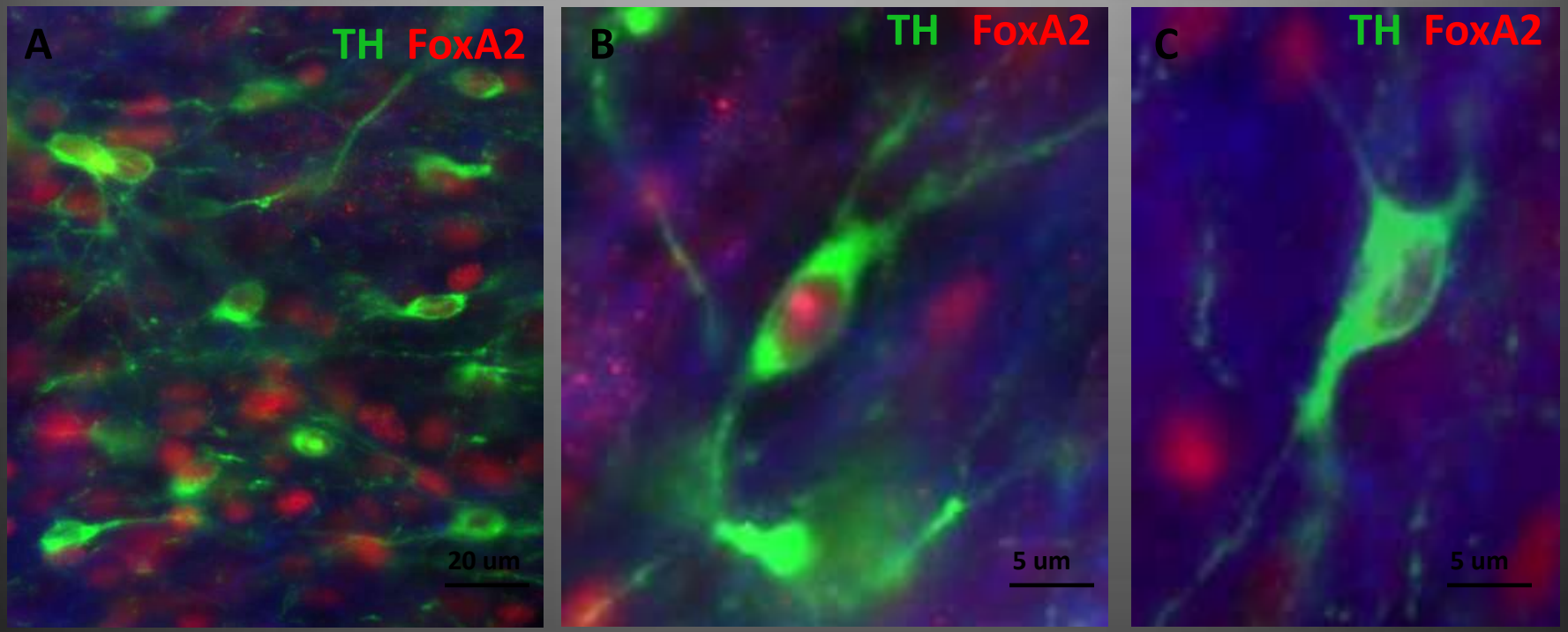
HESC-DA MPTP Rhesus-1



HESC-DA MPTP Rhesus-2

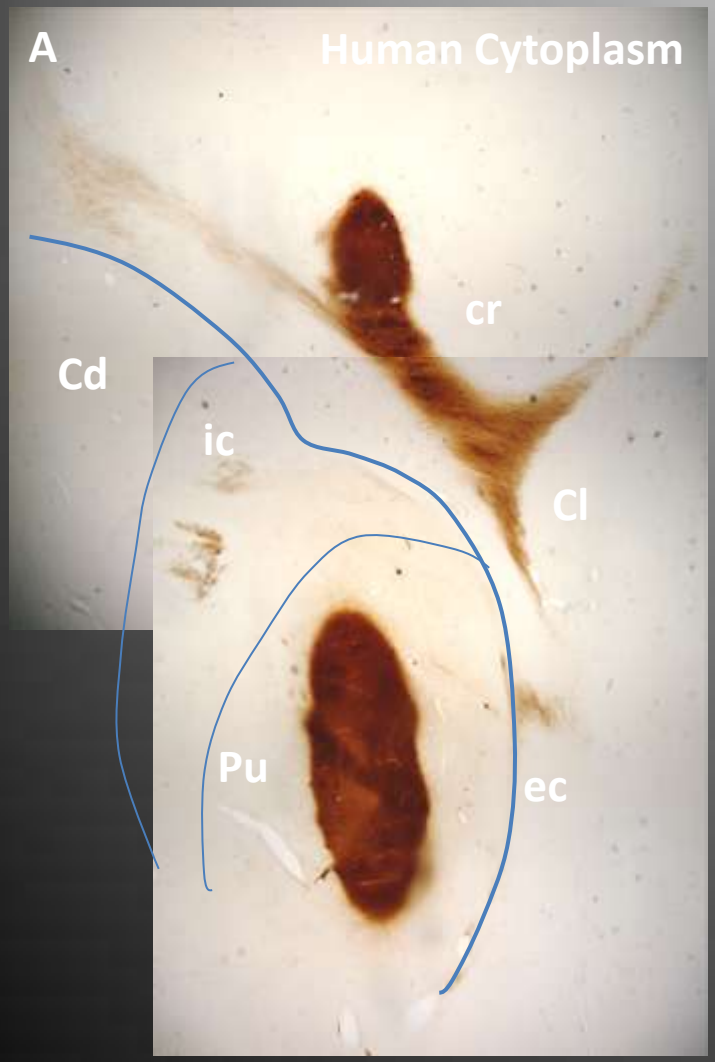
Transplanted HESC-DA Cells Are Similar to Fetal VM Dopaminergic Phenotype

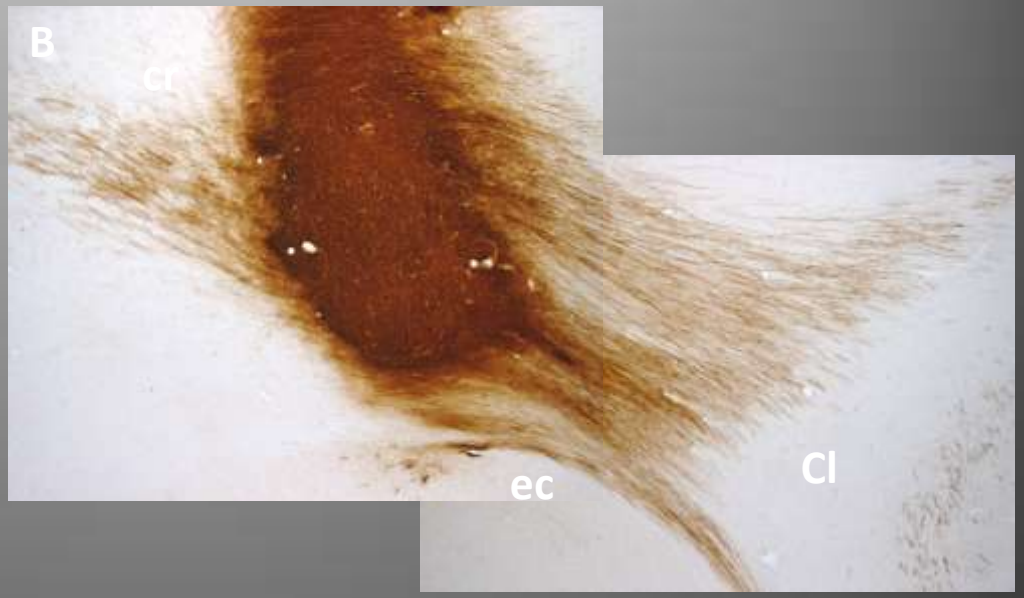
Midbrain Phenotype & Cell Morphology



Transplanted HESC-DA Cells Express Midbrain Specific Transcription Factor FoxA2

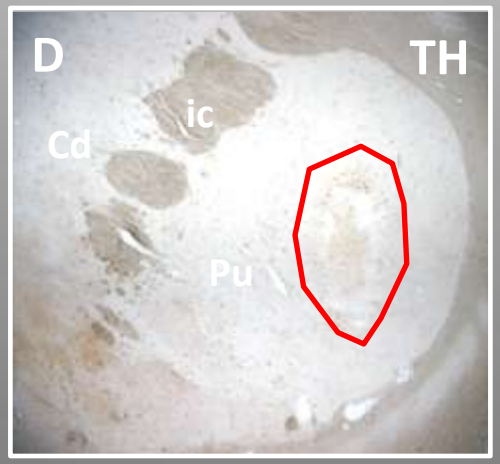
FP-Derived Human DA Neurons in PD Monkey (3-Month)

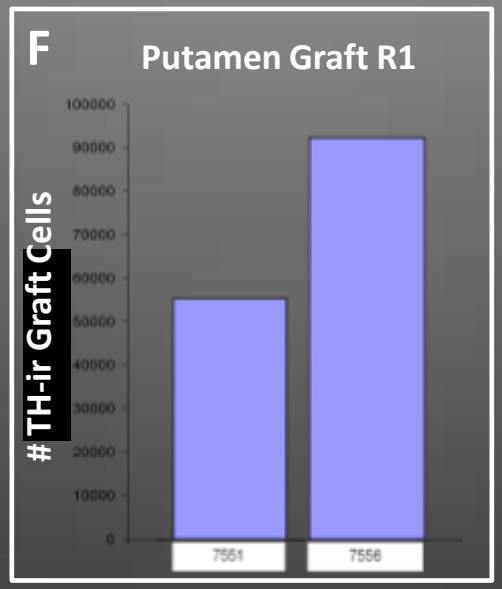




Transplanted Human Cells Survive and Project Fibers in MPTP Rhesus Monkey

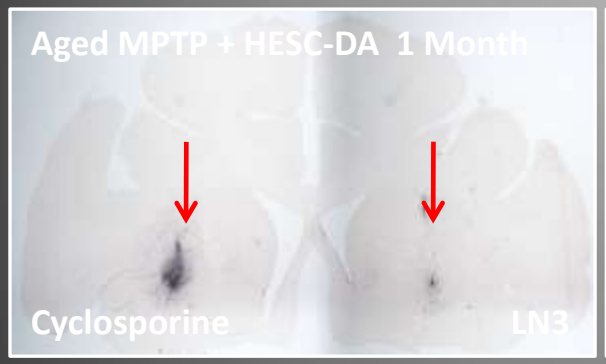
ESC-Derived Human Dopamine Neurons in PD Monkey

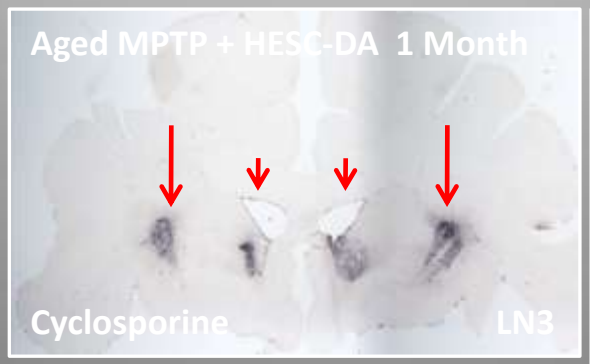


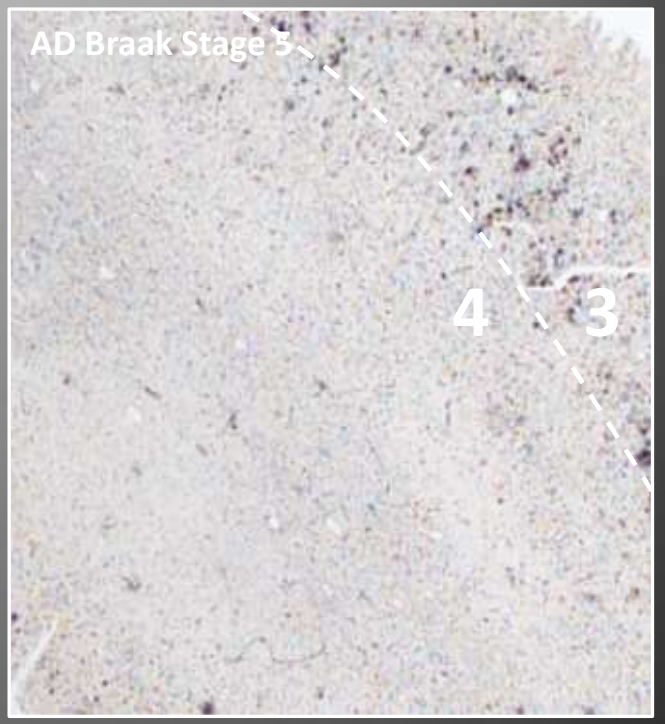


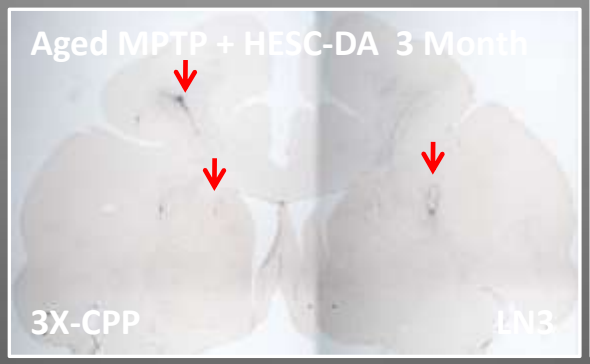
Transplanted Neurons Retain Dopaminergic Characteristics Up to 3-Months

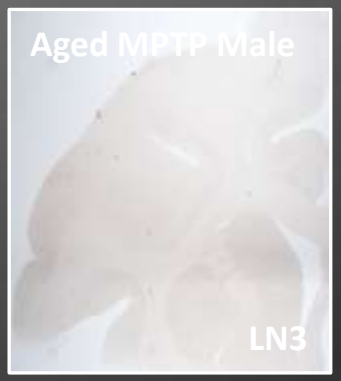
Host Immune Reaction (LN3) to Grafted HESC-DA

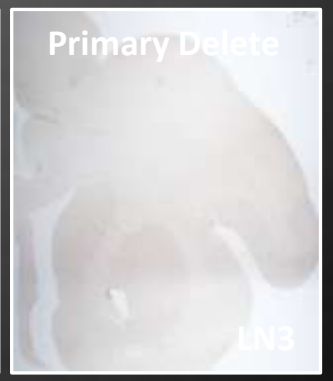






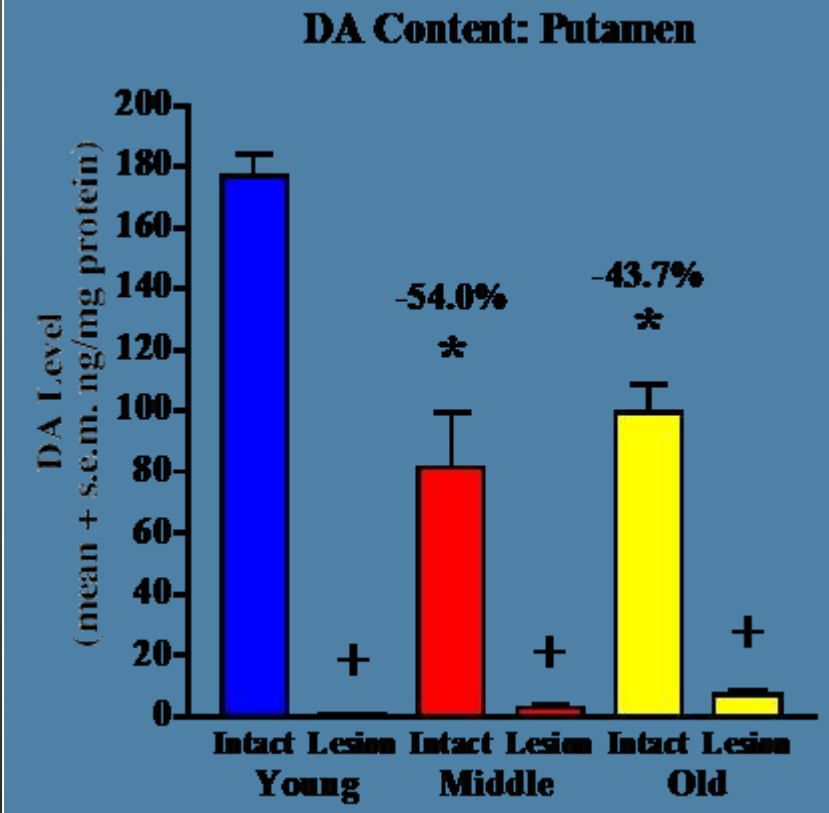


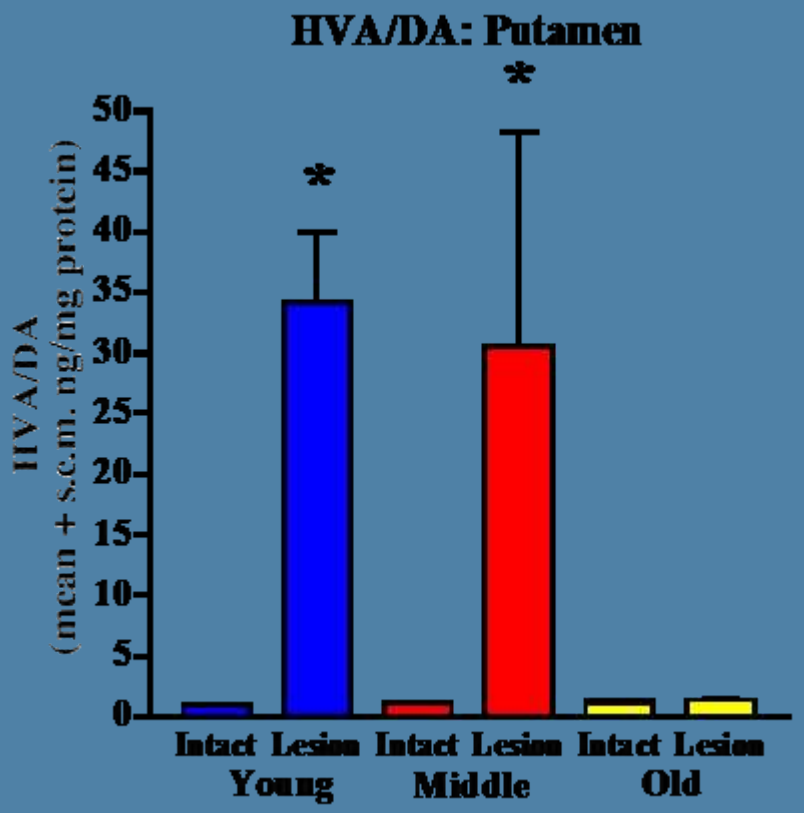


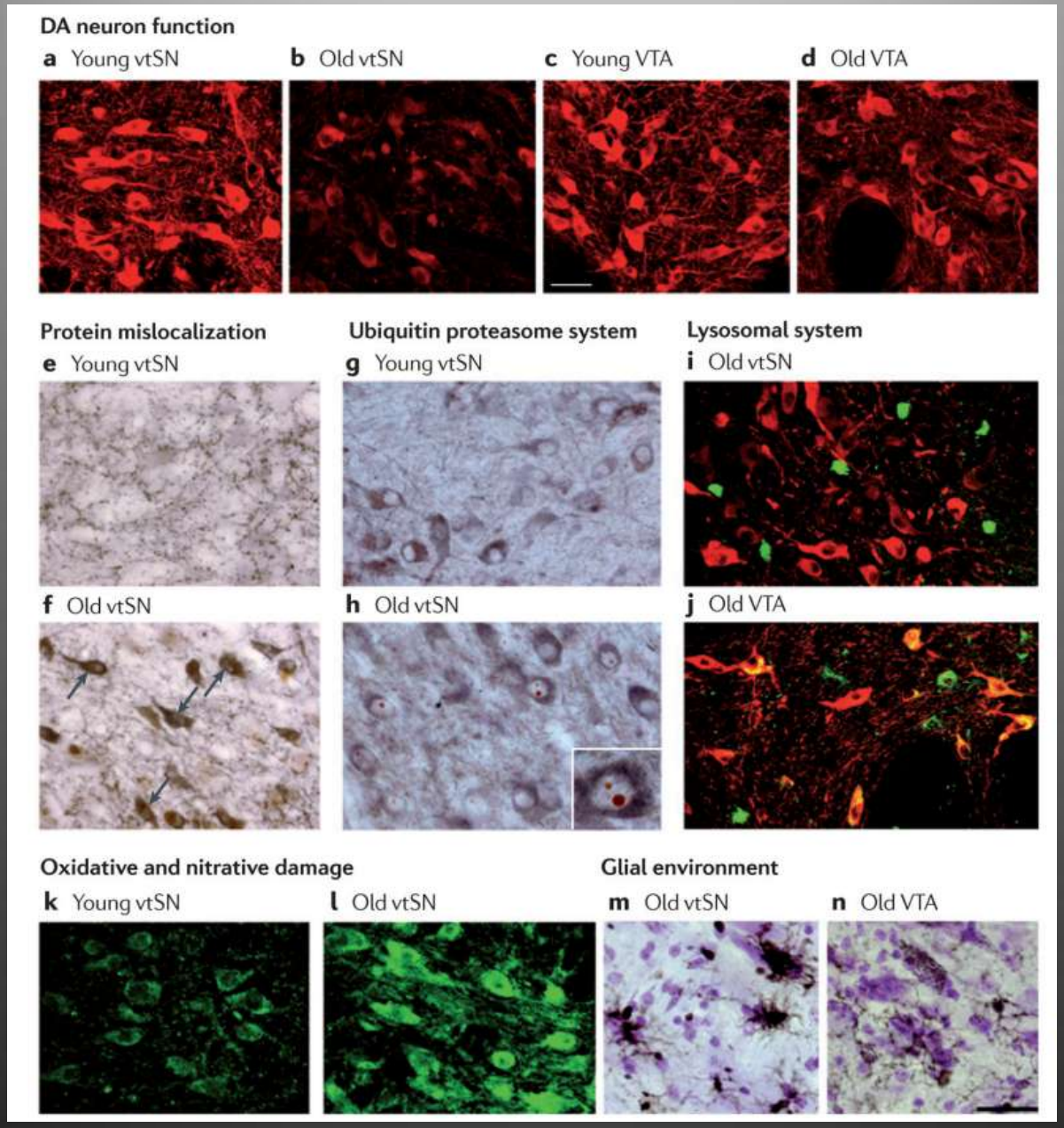


Transplanted HESC-DA Cells Induce Host Immune Reaction in MPTP Rhesus Monkey

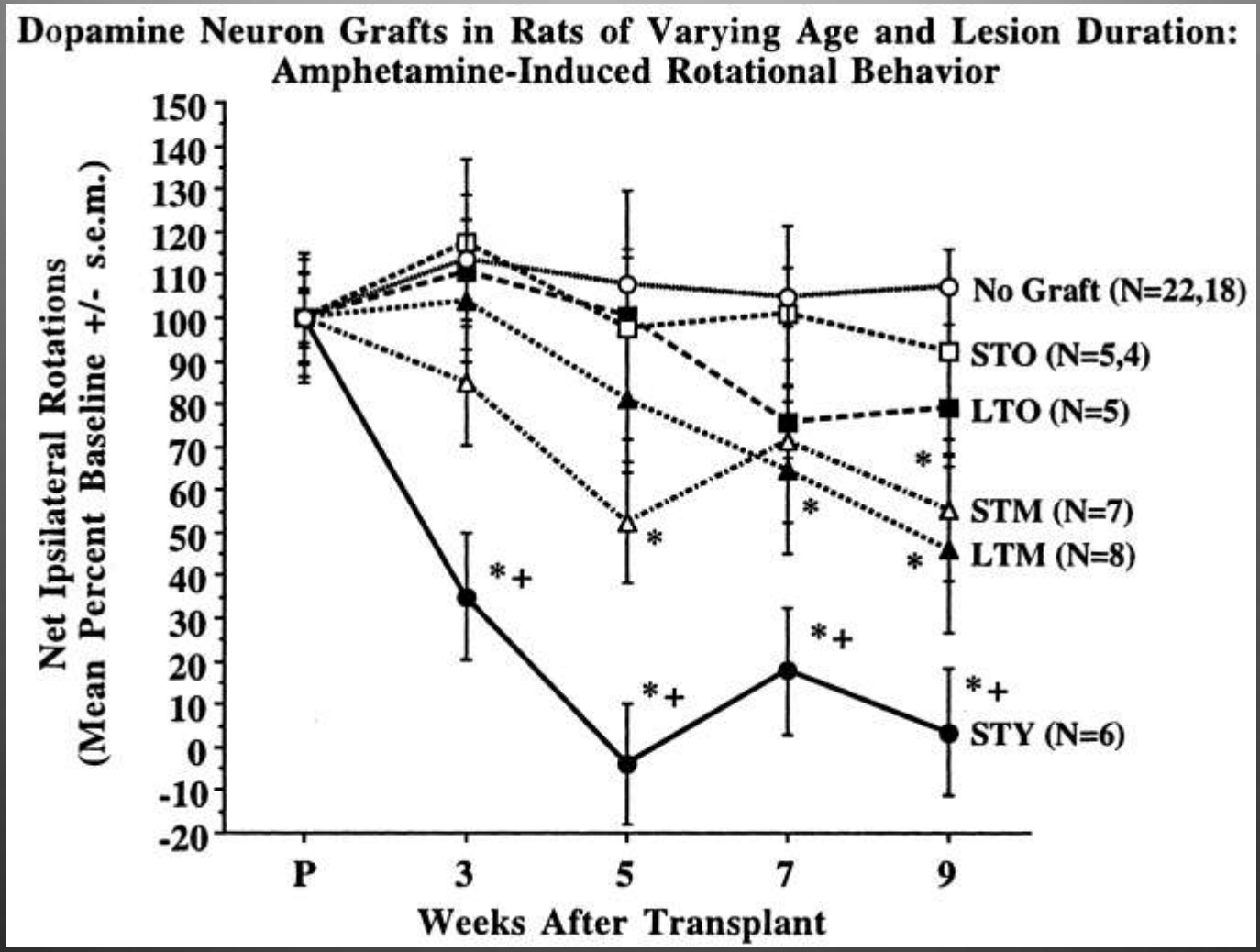
AGED MONKEYS AS A MODEL OF EARLY PD


DA Content: Putamen
DA Level
(mean + s.e.m. ng/mg protein)
200
180
160
140
120
100
80
60
40
20
0
-54.0%
*
-43.7%
*
+
+
+
Intact
Lesion
Intact
Lesion
Intact
Lesion
Young
Middle
Old


HVA/DA: Putamen
HVA/DA
(mean + s.e.m. ng/mg protein)
50
45
40
35
30
25
20
15
10
5
0
*
*
Intact
Lesion
Intact
Lesion
Intact
Lesion
Young
Middle
Old

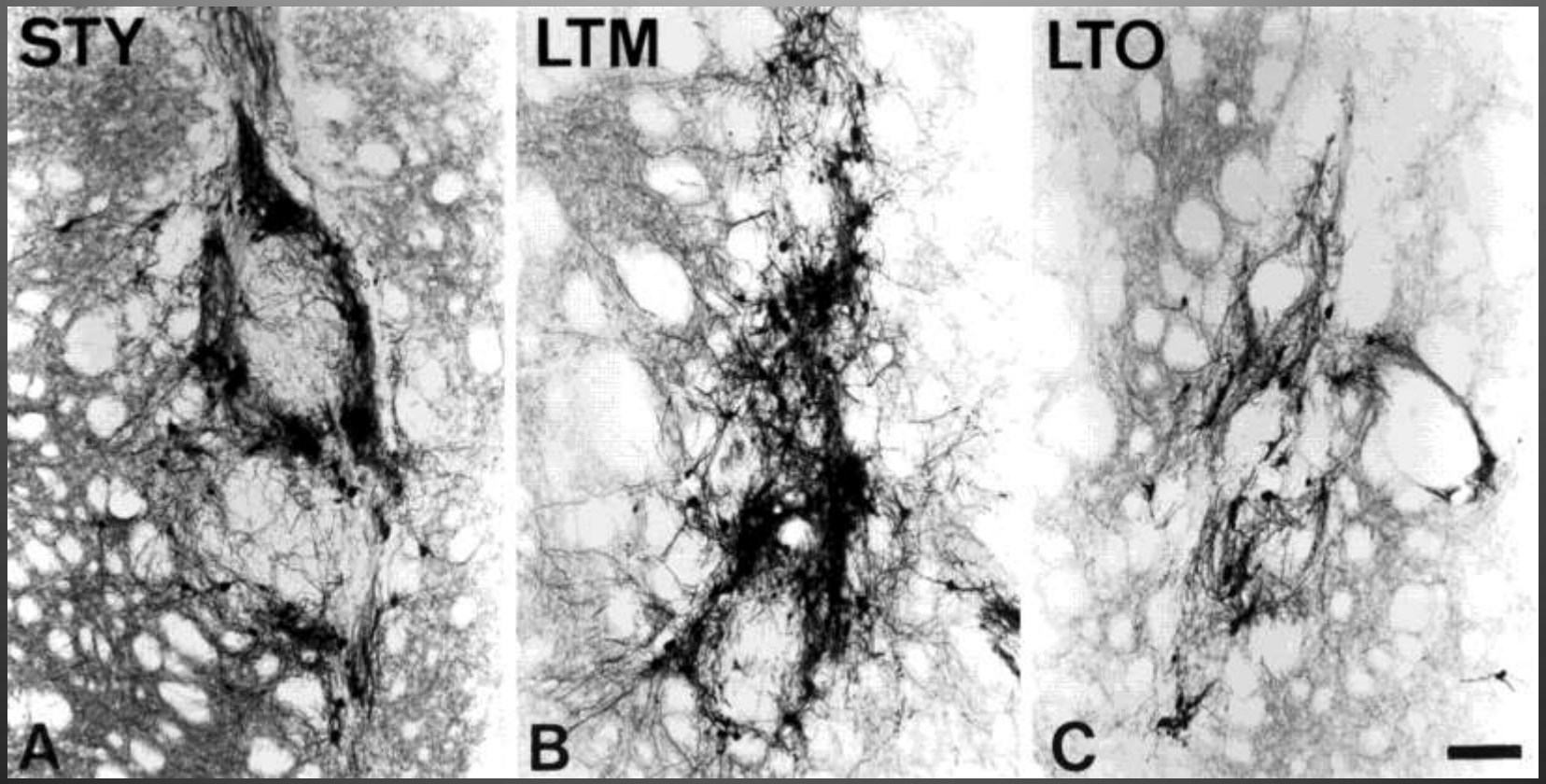

DA neuron function
a Young vtSN
b Old vtSN
c Young VTA
d Old VTA
Protein mislocalization
e Young vtSN
f Old vtSN
Ubiquitin proteasome system
g Young vtSN
h Old vtSN
Lysosomal system
i Old vtSN
j Old VTA
Oxidative and nitrative damage
k Young vtSN
l Old vtSN
Glial environment
m Old vtSN
n Old VTA

Amphetamine-induced rotational behavior in rats of varying age and lesion duration after implantation of DA neuron grafts.

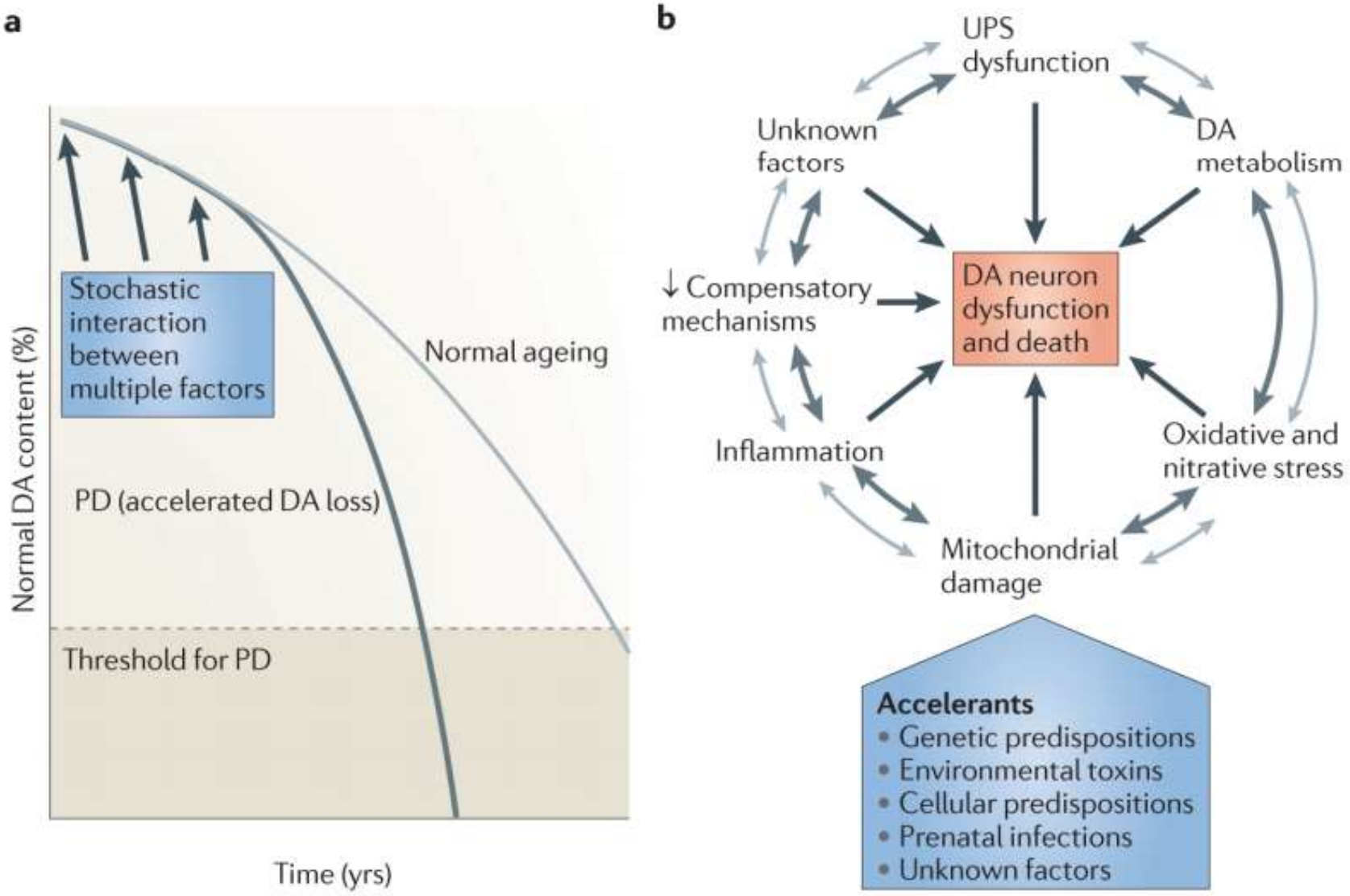


Collier T J et al. J. Neurosci. 1999;19:5563-5573

Representative ventral mesencephalic tissue grafts in rats of varying age and lesion duration.



Collier T J et al. J. Neurosci. 1999;19:5563-5573


a
Normal DA content (%)
Stochastic interaction between multiple factors
Normal ageing
PD (accelerated DA loss)
Threshold for PD
Time (yrs)
b
UPS dysfunction
Unknown factors
DA metabolism
↓ Compensatory mechanisms
DA neuron dysfunction and death
Inflammation
Oxidative and nitrative stress
Mitochondrial damage
Accelerants
Genetic predispositions
Environmental toxins
Cellular predispositions
Prenatal infections
Unknown factors

So where does cell replacement fit in the field of experimental therapeutics?

- Stem cell transplantations ultimate utility for PD may be a proof of principle for their ultimate use in other scenarios such as non-levodopa responsive and non-motor PD or in other neurodegenerative diseases (Huntington's disease??).

- Great strides have been made in the viability and appropriate phenotypic expression of grafted cells but the need to improve the pace and extent of fiber outgrowth remains.

The absence of a need to rely on the host system may put cell replacement in a competitive advantage when compared to gene therapy and trophic factor approaches that replay in the presence of a viable host system

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