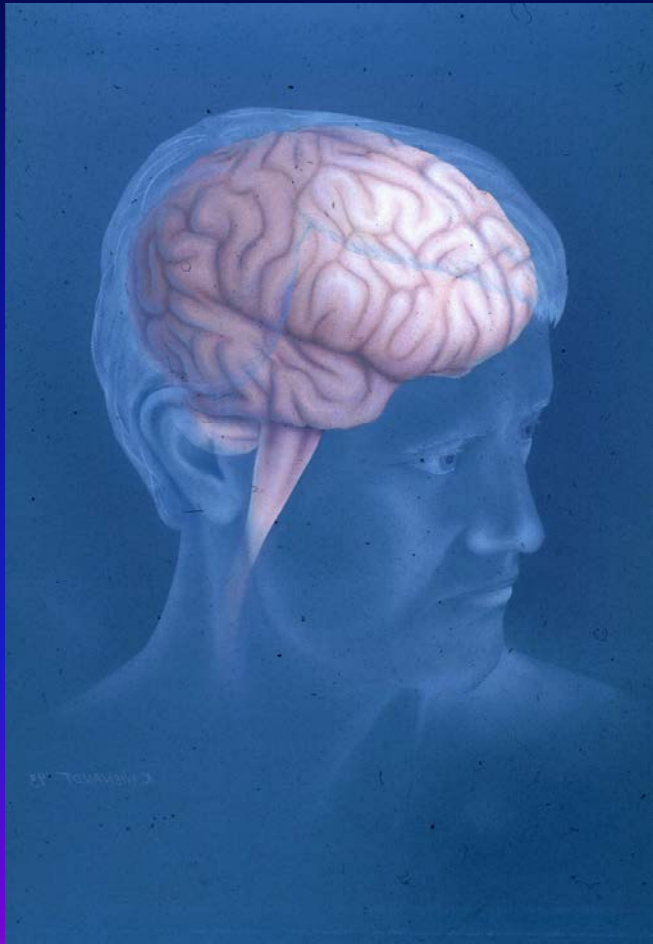


Gene Therapy for Parkinson's disease: Learning from my failures



Jeffrey H. Kordower, Ph.D.

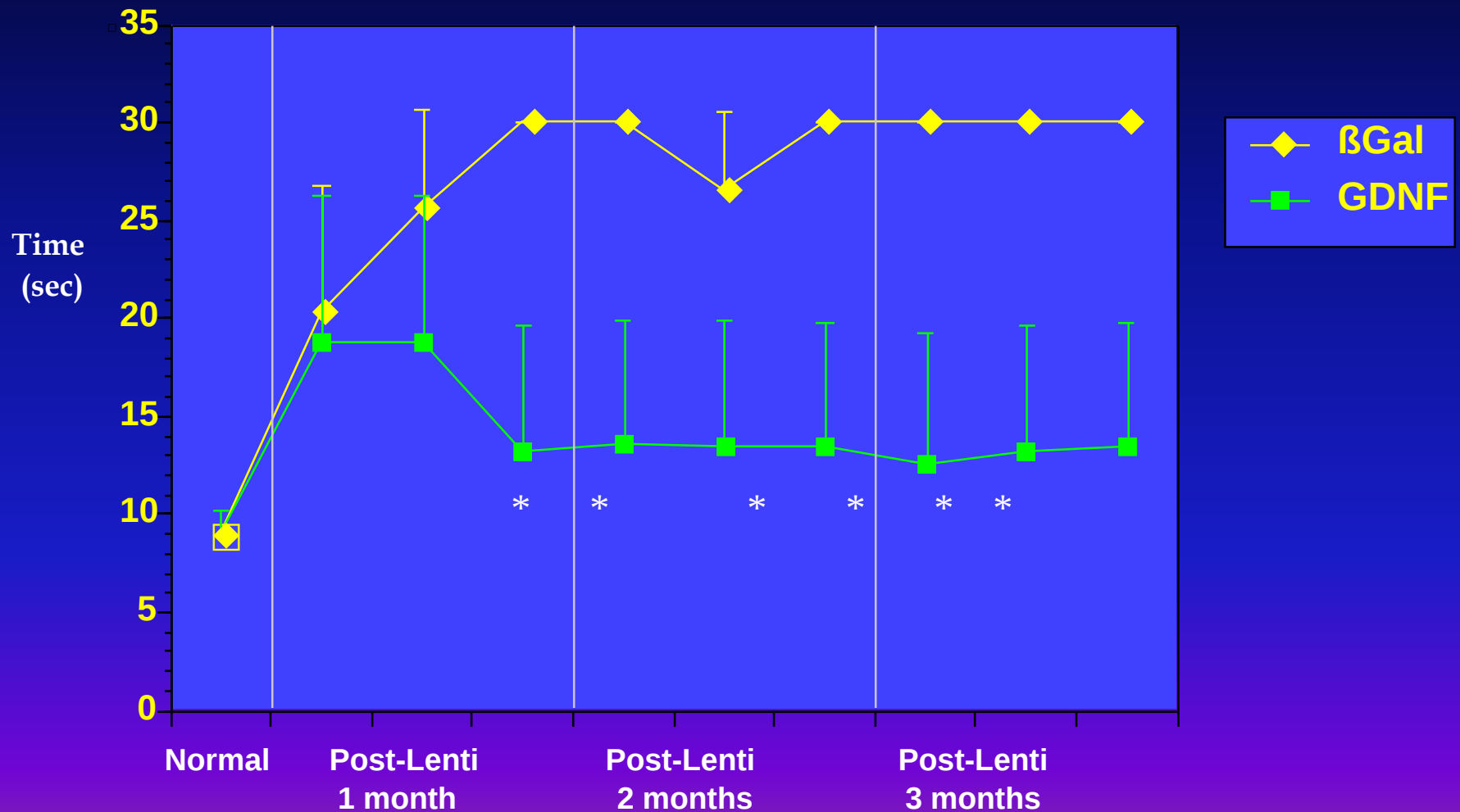
**The Alla V and Solomon Jesmer
Professor of Aging and
Neurological Sciences Director,
Research Center for Brain Repair
Rush University Medical Center**

My Clinical Trials for PD

- Adrenal Medullary Transplants
- Adrenal/peripheral nerve cogafts
- Fetal ventral midbrain transplants
- Intraputaminal neurturin gene therapy (Phase 1 and Phase II)
- Intraputaminal/intranigral neurturin gene therapy (Phase I and Phase II)



PREVENTION OF FINE-MOTOR DEFICITS IN MPTP-TREATED MONKEYS BY LENTIVIRAL GENE DELIVERY OF GDNF

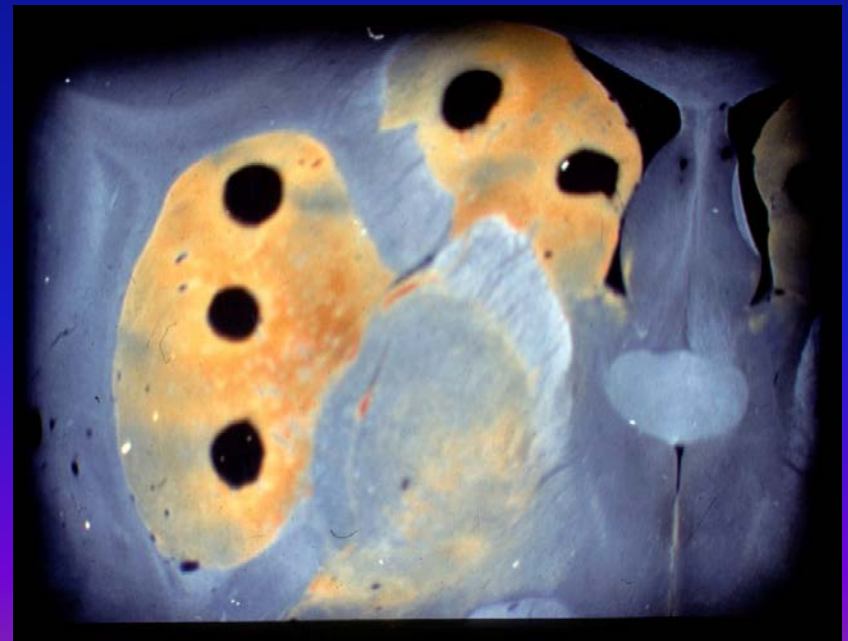


PROTECTION OF STRIATAL TH-IR BY LENTIVIRALLY DELIVERED GDNF IN MPTP-TREATED MONKEYS

Lenti- β Gal



Lenti-GDNF



PROTECTION OF NIGRAL NEURONS IN MPTP-TREATED MONKEYS BY LENTIVIRALLY-DELIVERED GDNF

Lenti- β Gal



Lenti-GDNF

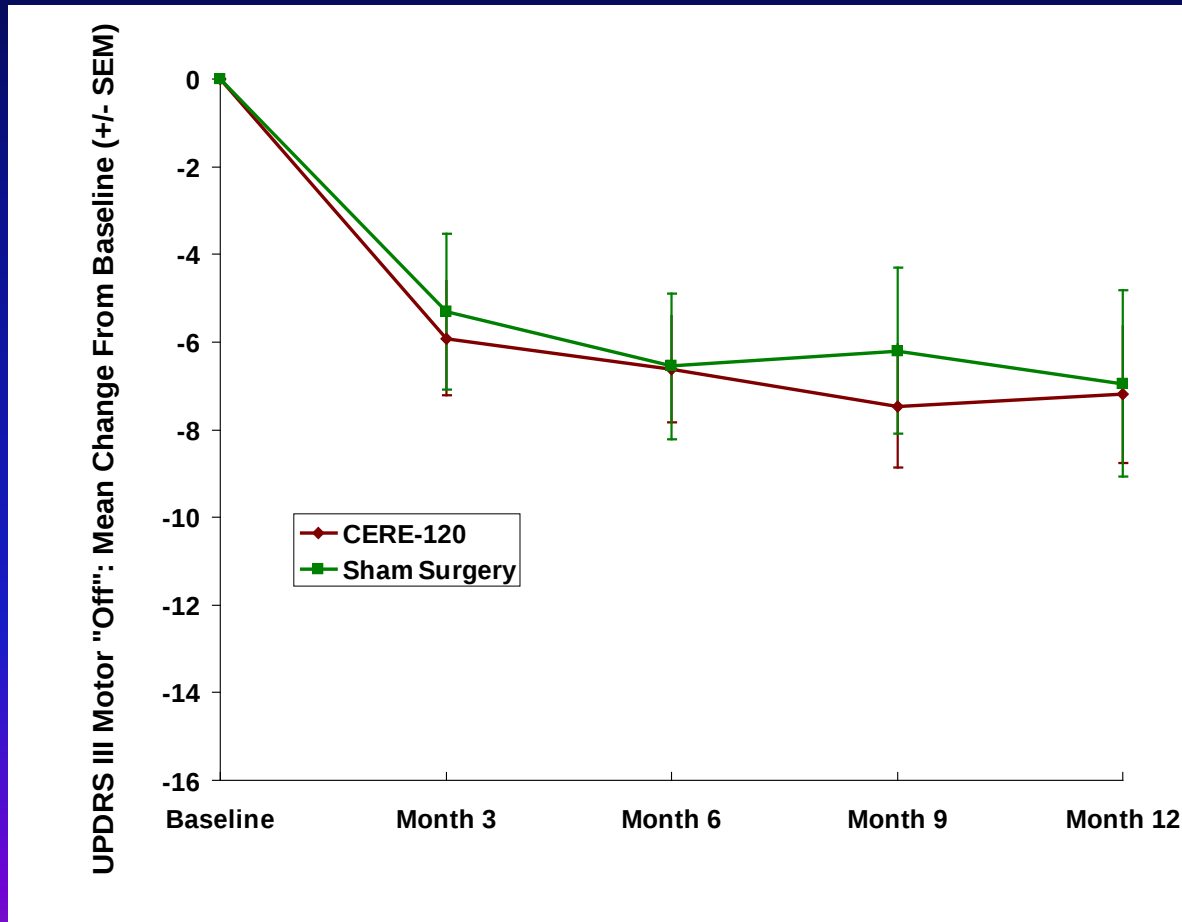


Success of GDNF and Neurturin in Animal Models of PD

- GDNF-MPTP treated mouse
 - GDNF-Aged rat
 - GDNF 6-OHDA lesioned rat
 - GDNF methamphetamine treated rat
 - GDNF-MPTP treated monkey
 - GDNF 6-OHDA treated monkey
 - GDNF aged monkey
 - GDNF axotomy
-
- NRTN-6-OHDA lesioned rat
 - NRTN-aged rat
 - NRTN-MPTP treated monkey
 - NRTN aged monkey

AAV2-Neurturin Gene Therapy in PD

Double Blind Study – 12 months



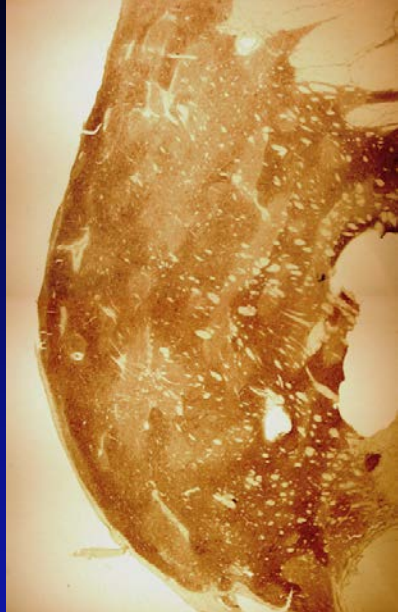


Where can you go wrong?

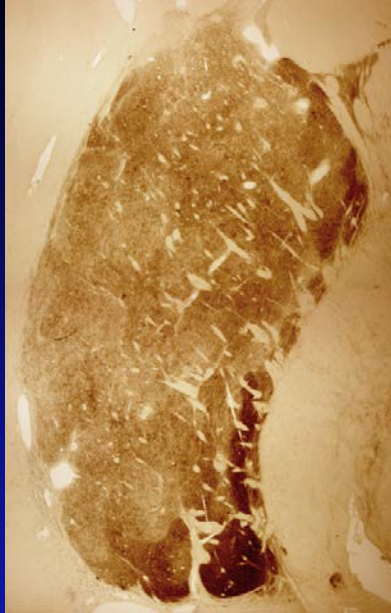
- Don't be so impressed with your preclinical data. Animals do not get Parkinson's disease.
- Do not design your preclinical studies to be successful. Design your preclinical studies appropriately to inform your clinical trials so THEY will be successful.
- Have a better understanding of the human disease.
- Evaluate your clinical data with rigor and honesty
- Give yourself a chance (with dosing and other critical parameters).

TH immunohistochemistry in the Putamen of human brain

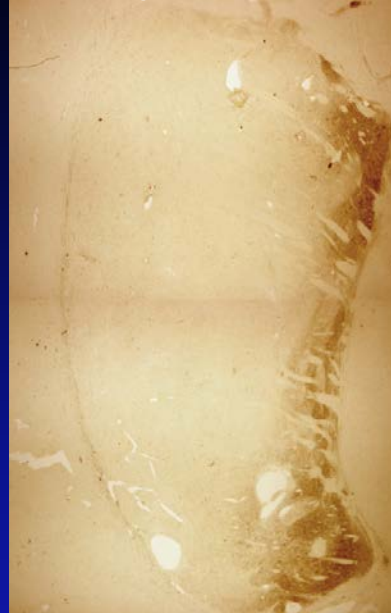
Control



3 years PD



4 years PD



5 years PD



11 years PD



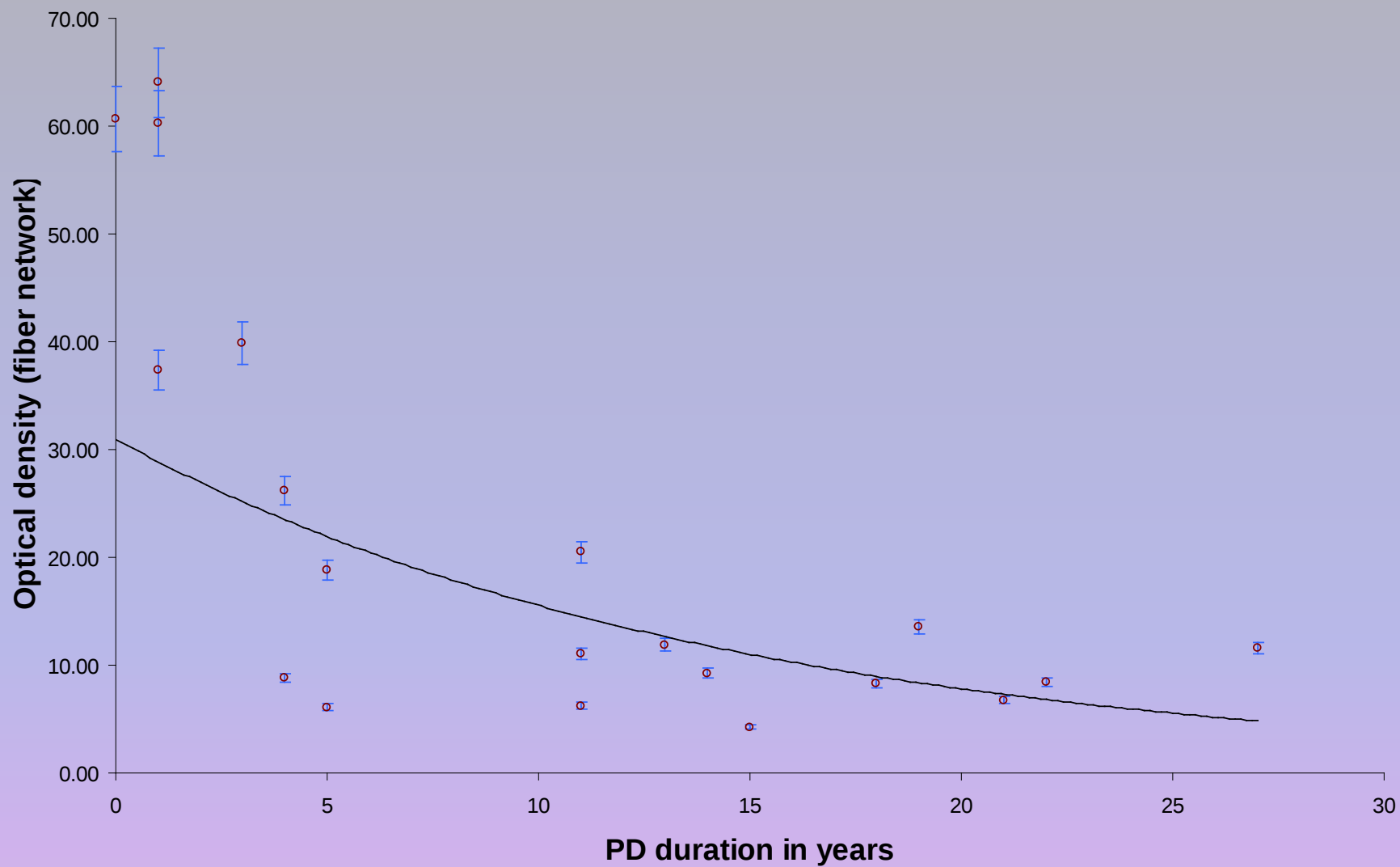
15 years PD



21 years PD

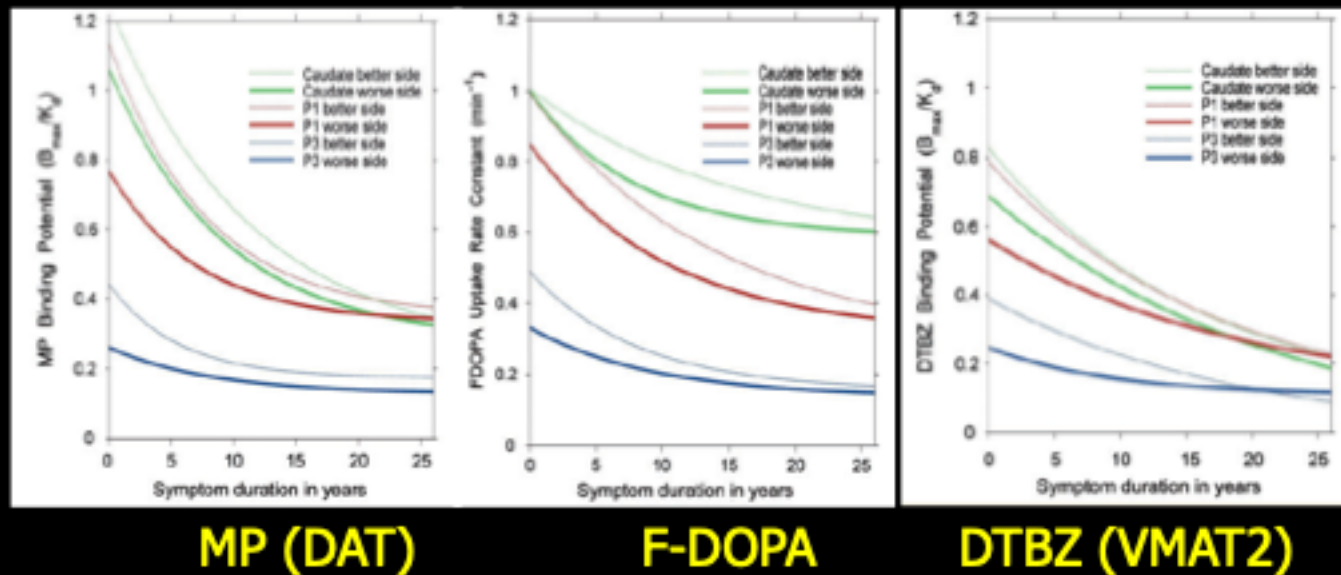


O.D. vs PD duration



Progression of sporadic PD is non-linear

Nandhagopal *et al.*, Brain 2009



Progressive parkinsonism in older adults is related to the burden of mixed brain pathologies

Aron S. Buchman, MD, Lei Yu, PhD, Robert S. Wilson, PhD, Sue E. Leurgans, PhD, Sukriti Nag, MD, PhD, Joshua L. Shulman, MD, PhD, Lisa L. Barnes, PhD, Julie A. Schneider, MD, MS, and David A. Bennett, MD

Neurology[®] 2019;92:1–10. doi:10.1212/WNL.0000000000007315

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Dr. Buchman

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Abstract

Objective

To examine whether indices of Parkinson disease (PD) pathology and other brain pathologies are associated with the progression of parkinsonism in older adults.

Methods

We used data from decedents who had undergone annual clinical testing prior to death and structured brain autopsy. Parkinsonism was based on assessment with a modified Unified Parkinson's Disease Rating Scale and a clinical diagnosis of PD was based on medical history. We used a series of mixed-effects models controlling for age and sex to investigate the association of PD pathology (nigral neuronal loss and Lewy bodies) and indices of 8 other brain pathologies with the progression of parkinsonism prior to death.

Results

During an average of 8.5 years' follow-up, more than half (771/1,430, 53.9%) developed parkinsonism proximate to death. On average, parkinsonism was progressive (estimate 0.130, SE 0.005, $p < 0.001$) in all older adults, but more rapid in adults with a clinical diagnosis of PD ($n = 52$; 3.6%) (estimate 0.066, SE 0.021, $p < 0.001$). Progression of parkinsonism was more rapid in adults with PD pathology (estimate 0.087, SE 0.013, $p < 0.001$). Alzheimer disease and several cerebrovascular pathologies were all independently associated with more rapid progression (all p values < 0.05). The association between a higher person-specific weighted pathology score and more rapidly progressive parkinsonism did not differ between individuals with and without a clinical diagnosis of PD (estimate 0.003, SE 0.047, $p = 0.957$).

Conclusion

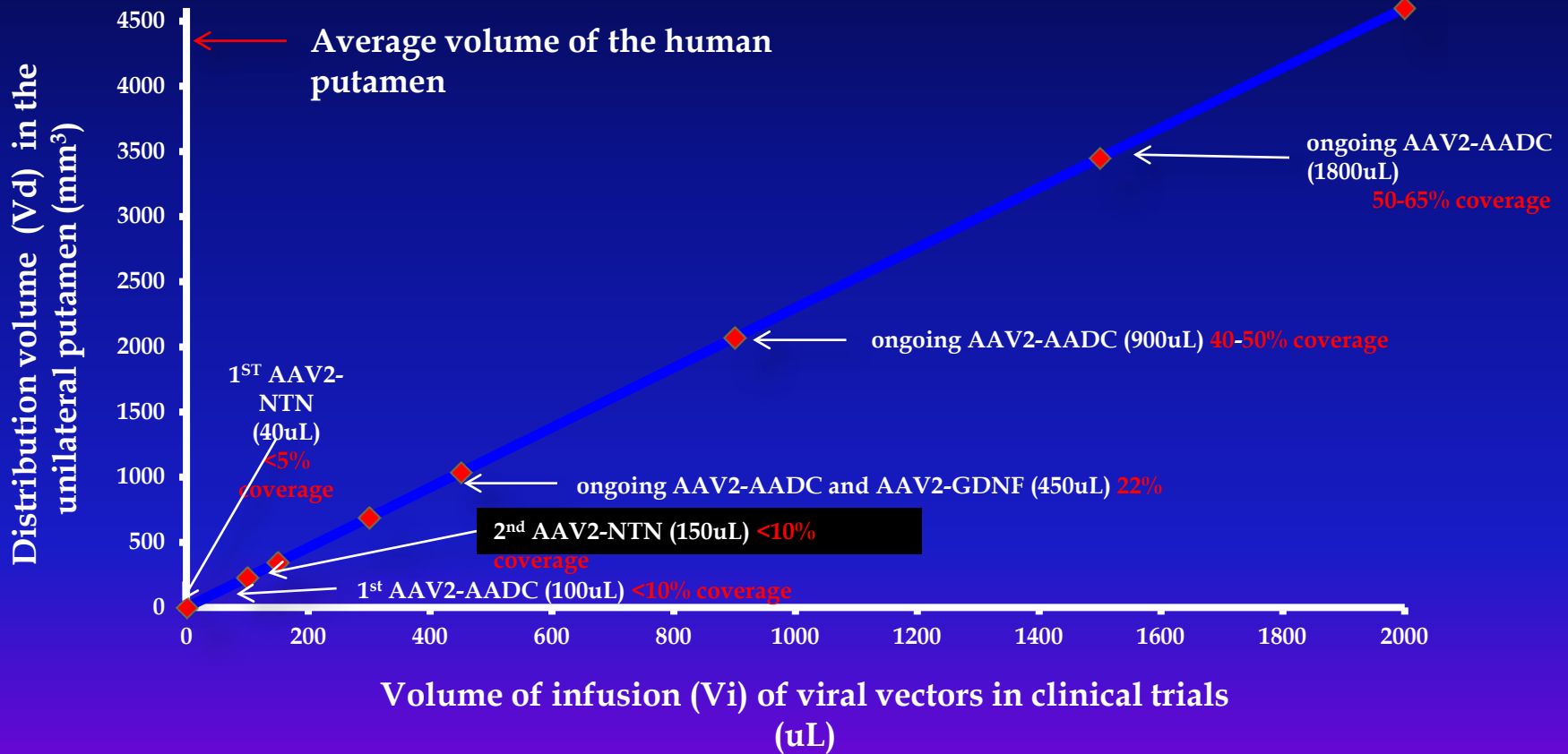
The rate of progressive parkinsonism in older adults with and without a clinical diagnosis of PD is related to the burden of mixed brain pathologies.

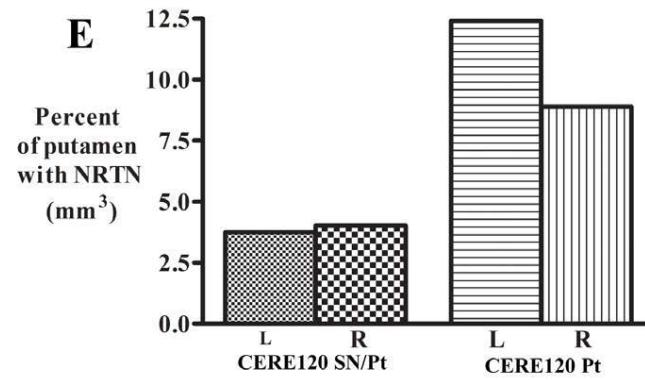
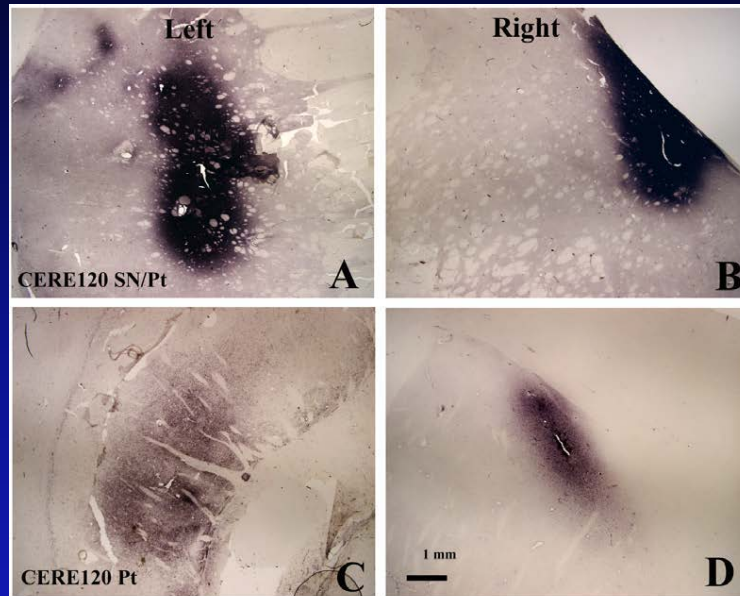
Lessons learned over the years with gene therapy trials in the brain:

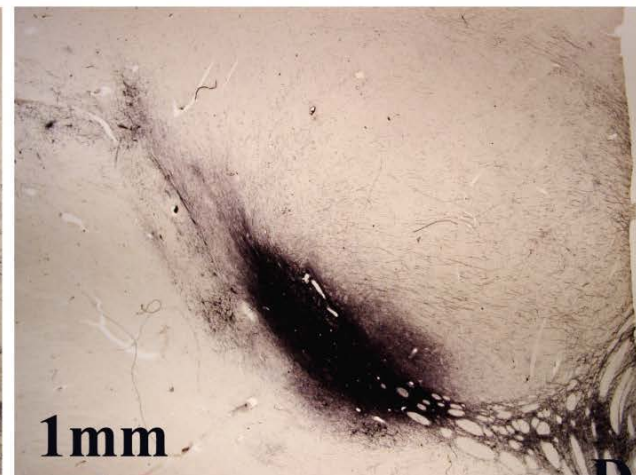
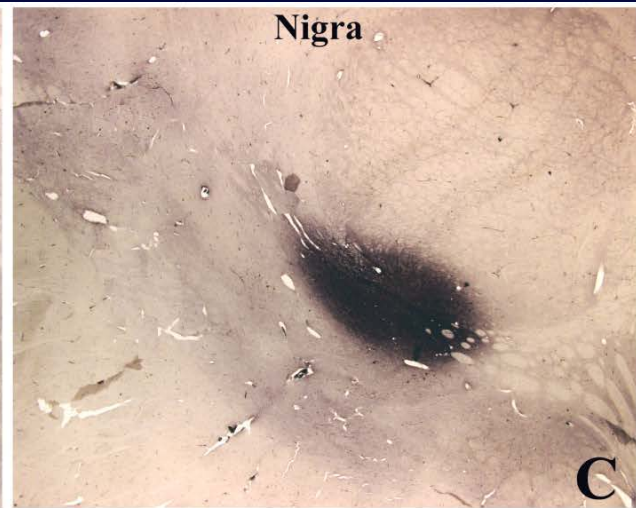
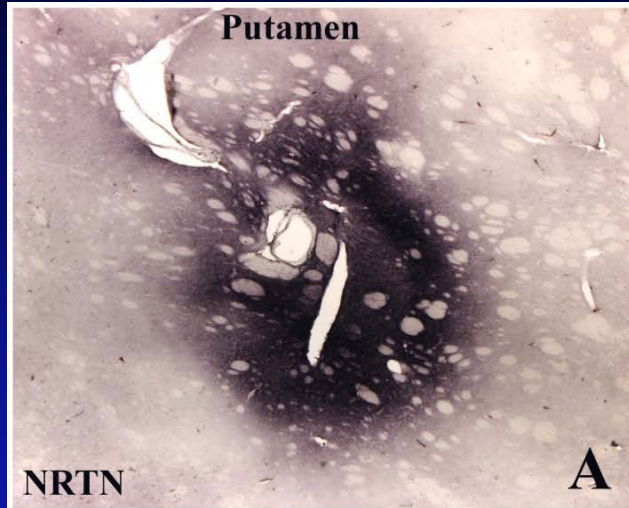
1. Delivery
2. Delivery
3. Delivery
4.



Success of gene transfer in PD putamen is volume dependent







The Big White Elephant in the Room

Lentiviral nigral delivery of GDNF does not prevent neurodegeneration in a genetic rat model of Parkinson's disease

C. Lo Bianco, N. Déglon, W. Pralunga and P. Aebischer

Viral delivery of glial cell line-derived neurotrophic factor (GDNF) currently represents one of the most promising neuroprotective strategies for Parkinson's Disease (PD). However, the effect of this neurotrophic factor has never been tested in the newly available genetic models of PD based on the viral expression of mutated α -synuclein. In this study, we evaluated the ability of lentiviral vectors coding for GDNF (lenti-GDNF) to prevent nigral dopaminergic degeneration associated with the lentiviral mediated expression of the A30P mutant human α -synuclein (lenti-A30P). This virally based rat model develops a progressive and selective loss of dopamine neurons associated with the appearance of α -synuclein containing inclusions, thus recapitulating the major hallmarks of PD. Lenti-GDNF was injected in the substantia nigra 2 weeks before nigral administration of lenti-A30P. **Although a robust expression of GDNF was observed in the whole nigrostriatal pathway due to retrograde and/or anterograde transport, lenti-GDNF did not prevent the α -synuclein-induced dopaminergic neurodegeneration in the lentiviral-based genetic rat model of PD.** These results suggest that sustained GDNF treatment cannot modulate the cellular toxicity related to abnormal folded protein accumulation as mutated human α -synuclein.

Table.1. Severity of parkinsonian signs

Groups	Global-P	Gait	Bradykinesia	Rigidity	Tremor
Non-parkinsonism	6.51±3.40	3.29±1.87	3.37±3.29	5.00 ±5.5	4.04±3.0
parkinsonism	15.53±10.46 ###	36.94±22.41* *	14.65±12.33 ###	13.61±22.41 ###	3.29±5.46# ##
Parkinson's disease	37.50±10.80 ***	52.14±24.84* **	52.97±16.75* **	41.66±20.33* **	9.69±13.17 **

Global-P = global Parkinsonism; ### P<0.001 compared with Parkinson's disease;

** P<0.01 and ***P<0.001 compared with non-parkinsonism; Mean ± SD.

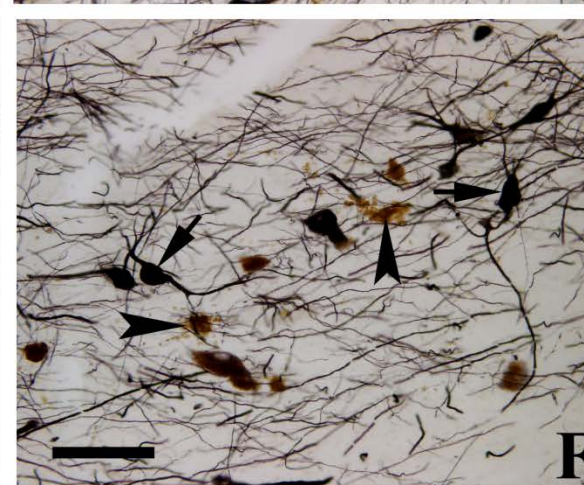
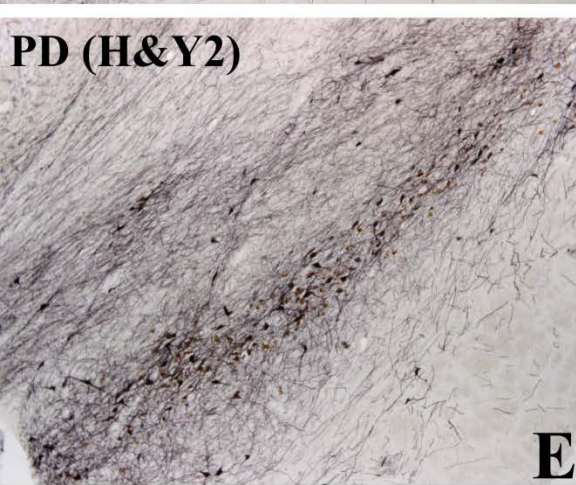
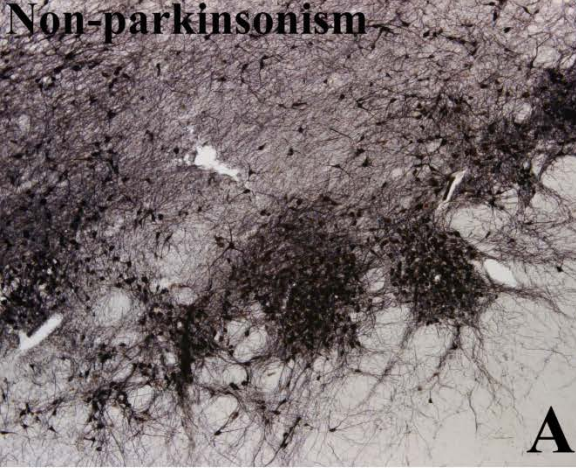


Fig. 2. Patterns of tyrosine hydroxylase (TH) immunoreactivity in substantia nigra from non-parkinsonism (A, B), parkinsonism (C, D), and H&Y stage 2 Parkinson's disease (PD(H&Y); E, F). There was a clear reduction of TH immunoreactivities in parkinsonism subjects (C, D) compared with non-parkinsonism (A, B). PD case (E) displayed severe reduction of TH immunoreactivity relative to parkinsonism (C). Some remaining nigral melanized neurons exhibited TH immunoreactivities (arrows; D, F) while others displayed no detectable TH immunoreactivity (arrowheads; D; F). Scale bar = 100 μ m in F (applies to B, D); 500 μ m for A, C, E.

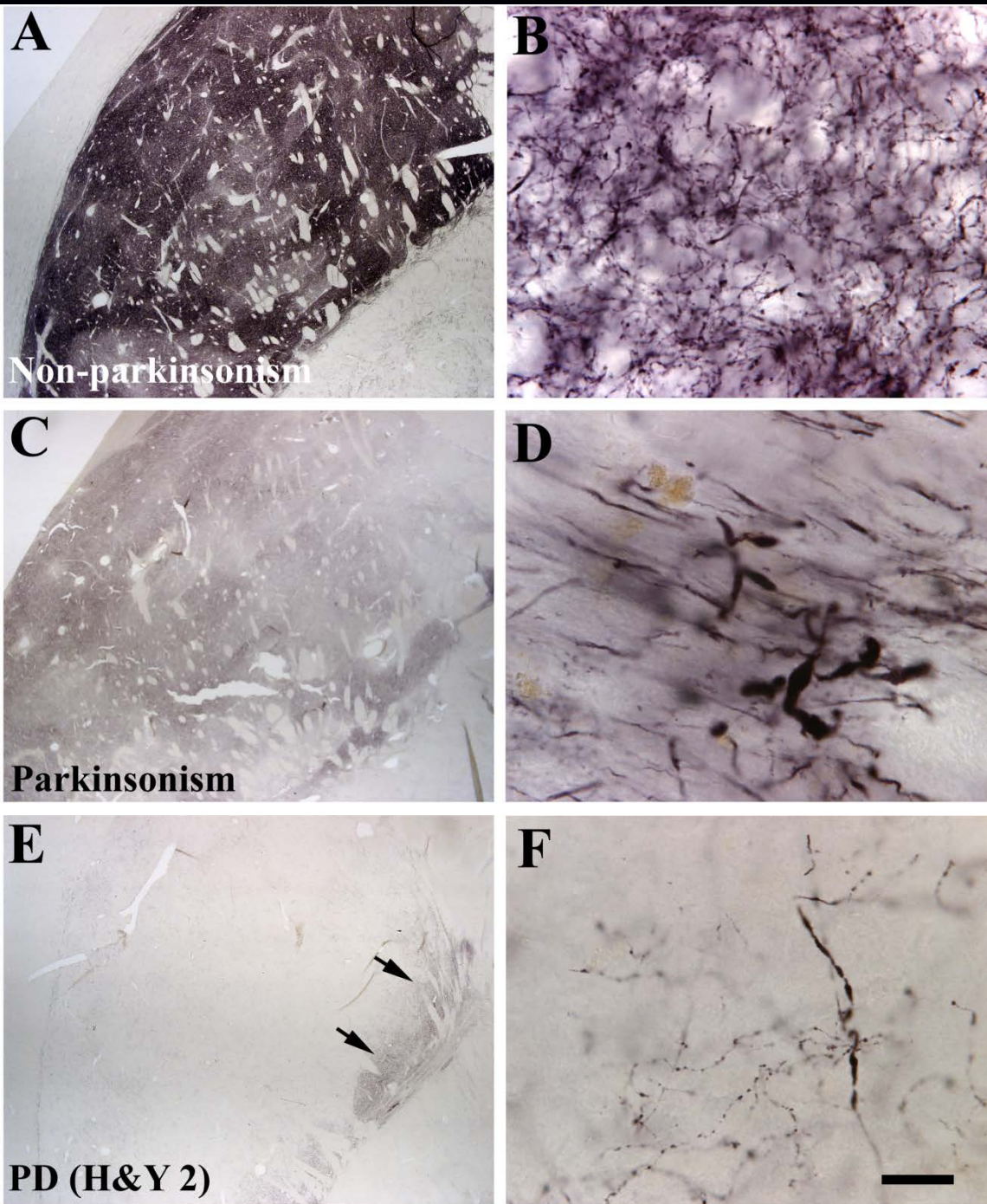


Fig. 3. Patterns of tyrosine hydroxylase (TH) immunoreactivities in putamen from non-parkinsonism (A, B), parkinsonism (C, D), and H&Y2 Parkinson's disease (PD(H&Y2); E, F). A severe reduction of tyrosine hydroxylase immunoreactivities was observed in parkinsonism (C) relative to non-parkinsonism (A). PD patient displayed undetectable TH immunoreactivity in major putamen, except the ventromedial putamen near globus pallidus (arrows; E). At higher magnification, dense TH immunoreactive fine fibre was observed in non-parkinsonism (B). At contrast, the TH labeled fibres were dramatically decreased and some of remaining TH immunopositive fibres displayed swollen varicosities and segments in parkinsonism (D). In PD case, remaining TH immunoreactive fibre exhibited swollen segments (F). Scale bar = 20 μ m in F (applies to B and D); 2.0mm for A, C, and E.

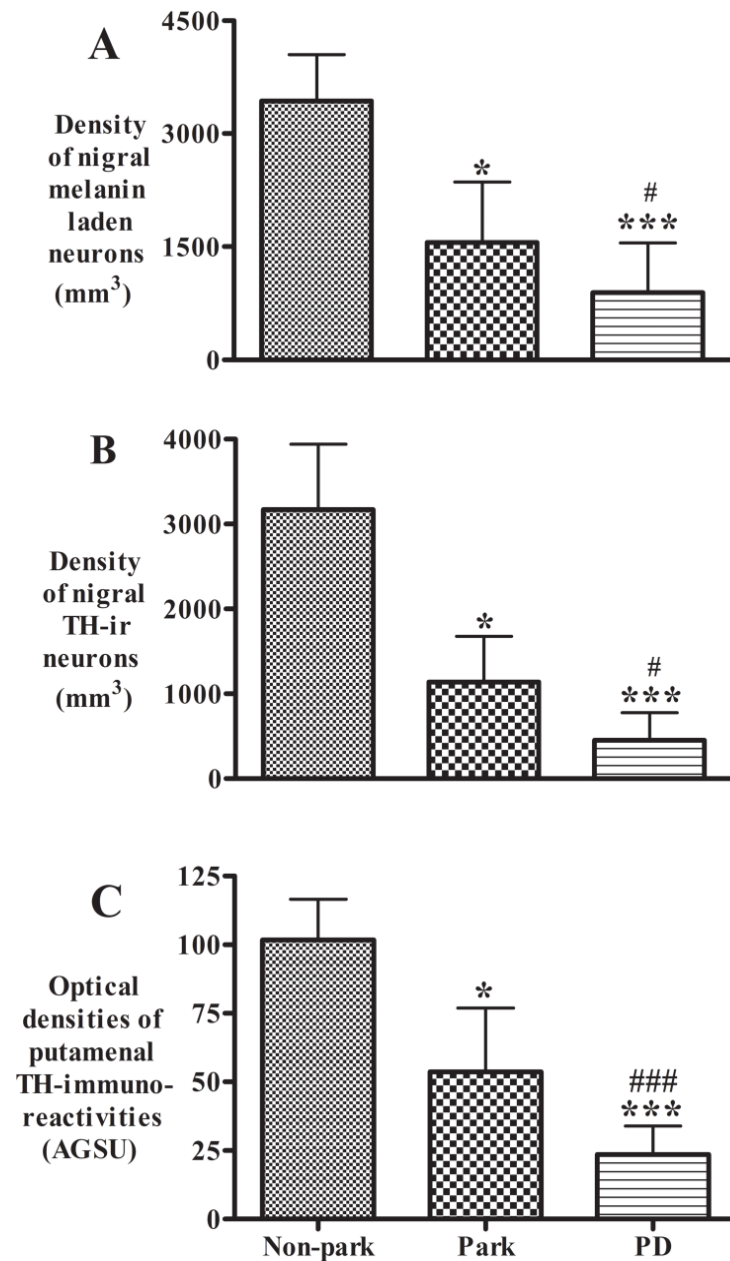


Fig. 4. Histograms showing the densities of nigral melanin-laden neurons (A) and nigral tyrosine hydroxylase (TH) immunoreactive neurons (B) and optic densities of TH immunoreactivities in putamen (C) from subjects with non-parkinsonism (Non-park) and parkinsonian (Park), and patients with Parkinson's disease (PD). The densities of nigral melanin-laden and TH-immunoreactive neurons and the levels of putamenal TH immunoreactivities were significantly reduced in parkinsonism as compared with non-parkinsonism. There were statistic difference between parkinsonism and PD. (* <0.05 , *** $P<0.001$ compared with non-parkinsonism; # $p<0.05$, ### $P<0.001$ compared with parkinsonism). Data are mean $\pm$ SD. AGSU = arbitrary grey scale units.

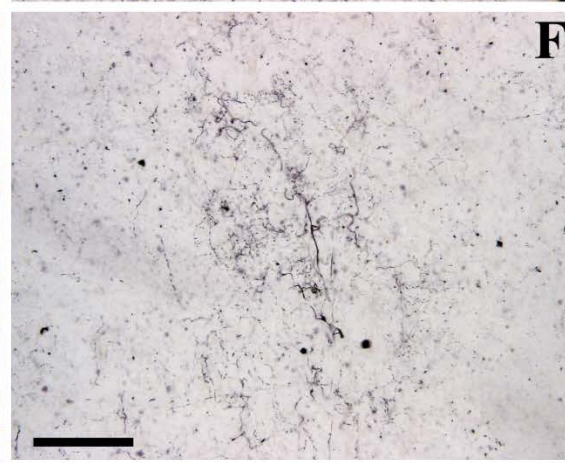
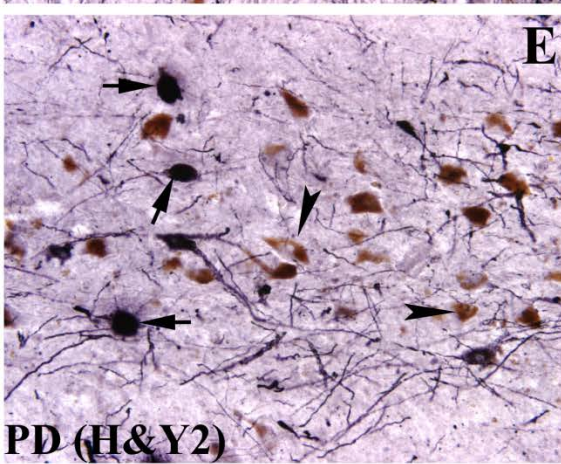
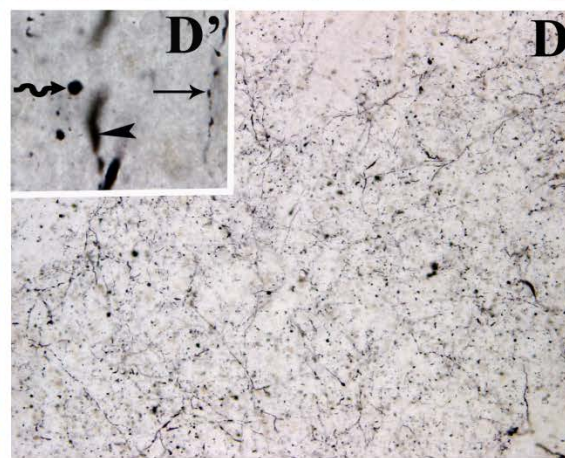
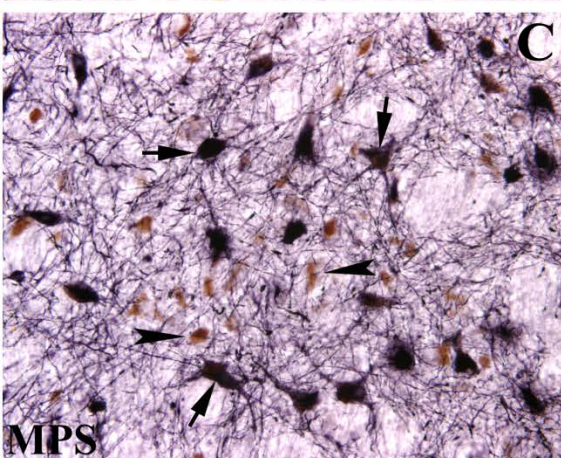
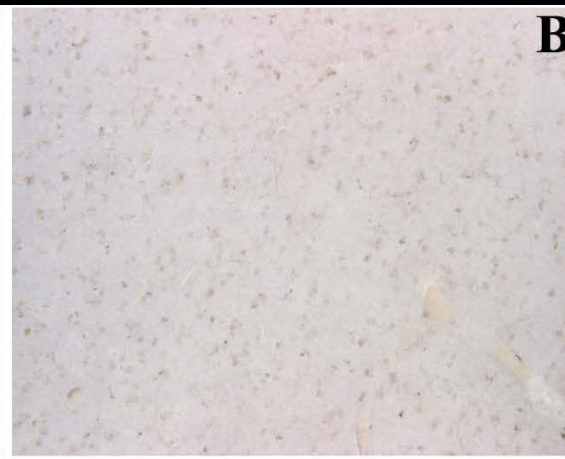
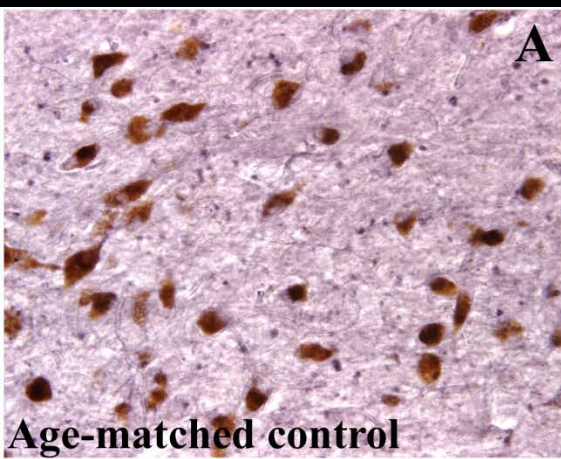
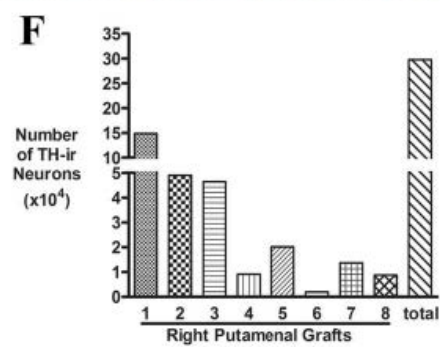
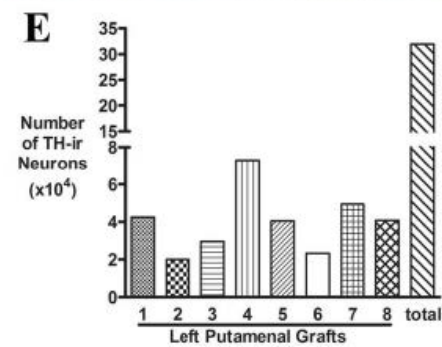
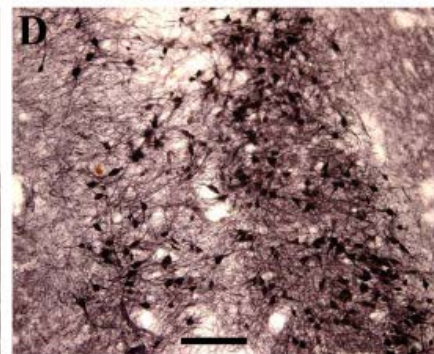
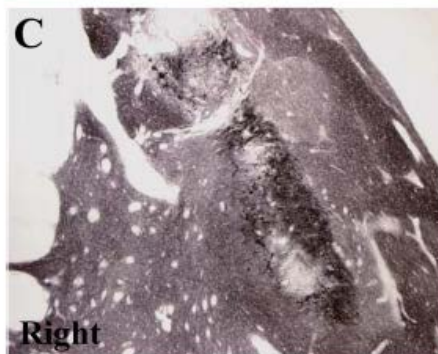
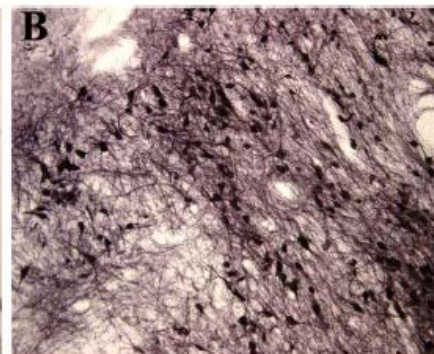
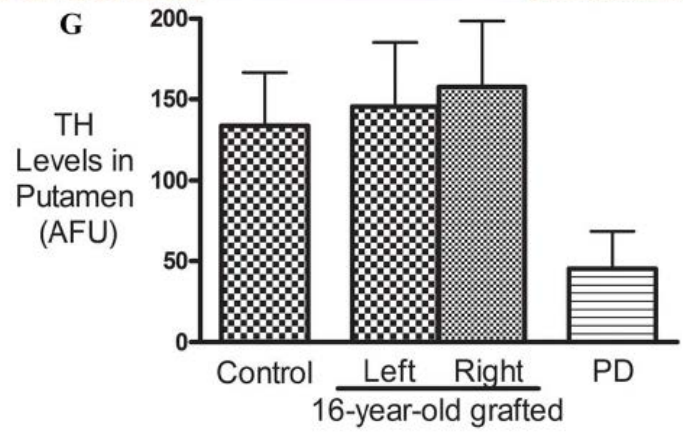
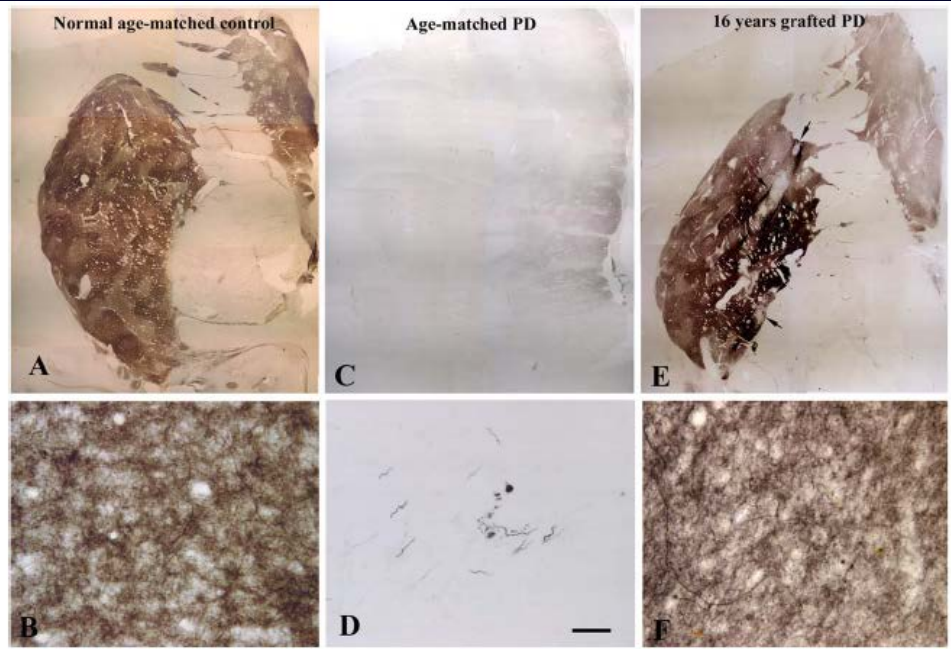
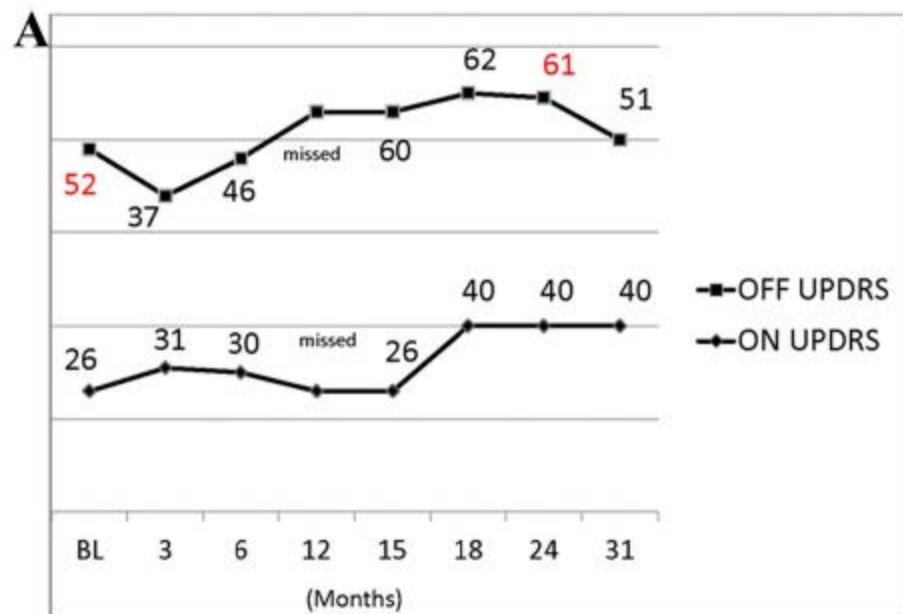


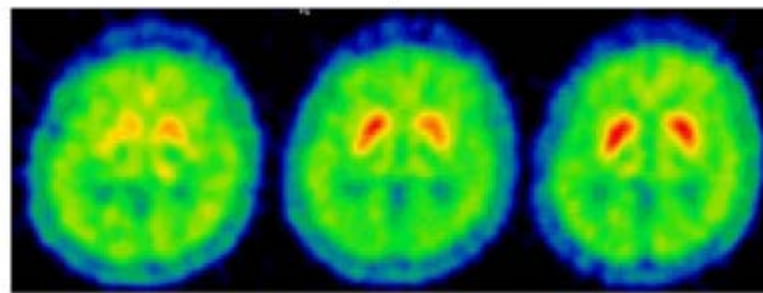
Fig. 5. Pattern of phosphorylated α -synuclein (p-S129- α -syn) immunoreactivities in substantia nigra (A, C, E) and putamen (B, D, D', F) from subjects with non-parkinsonism (A, B) and parkinsonian (C, D, D') and H&Y2 Parkinson's disease (PD(H&Y2); E, F). P-S129- α -syn immunoreactivity was undetectable in non-parkinsonism subjects (A, B). In contrast, p-S129- α -syn immunoreactive nigral neurons (C, E) and putamenal fibres (D, F) were observed in subjects with parkinsonism (C, D) and patients with PD (E, F). Note more p-S129- α -syn immunoreactive processes in subjects with parkinsonism (C, D) than patients with PD (E, F). The p-S129- α -syn immunoreactive processes displayed varicosities (arrow; D'), dot (curved arrow, D'), and segments (arrowhead, D') in putamen. Scale bar = 100 μ m in F (applies to A-D) and 20 μ m for D'.







B Fetal transplantation

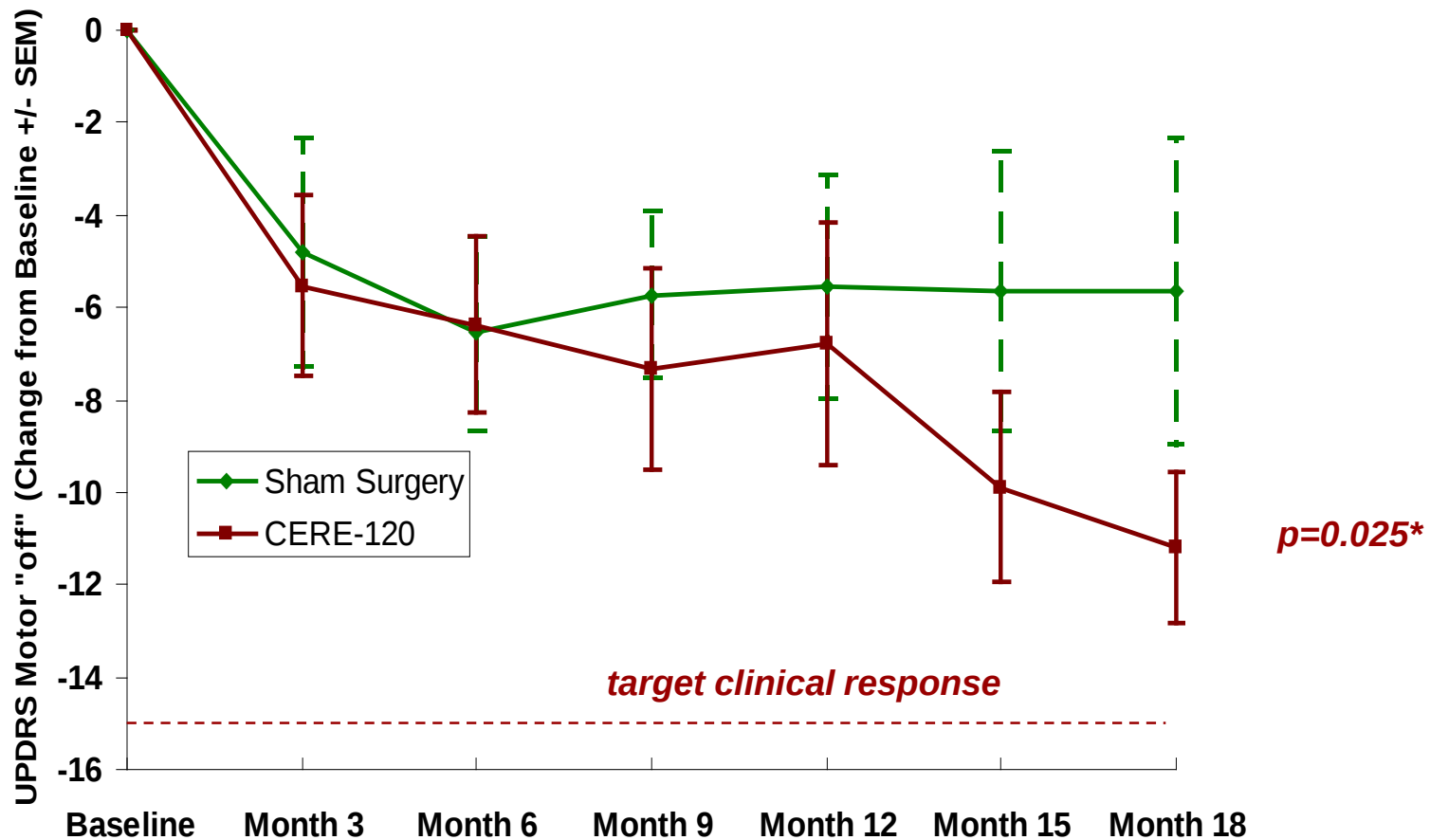


Baseline

One year

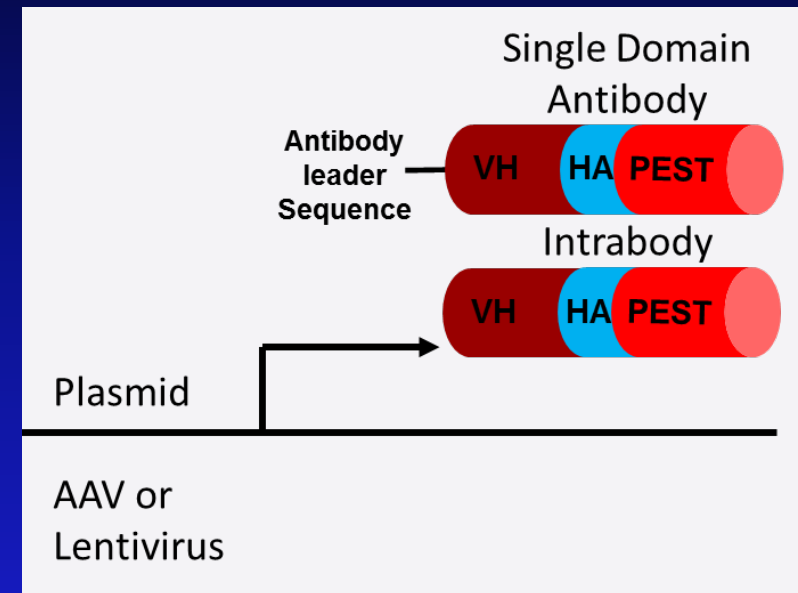
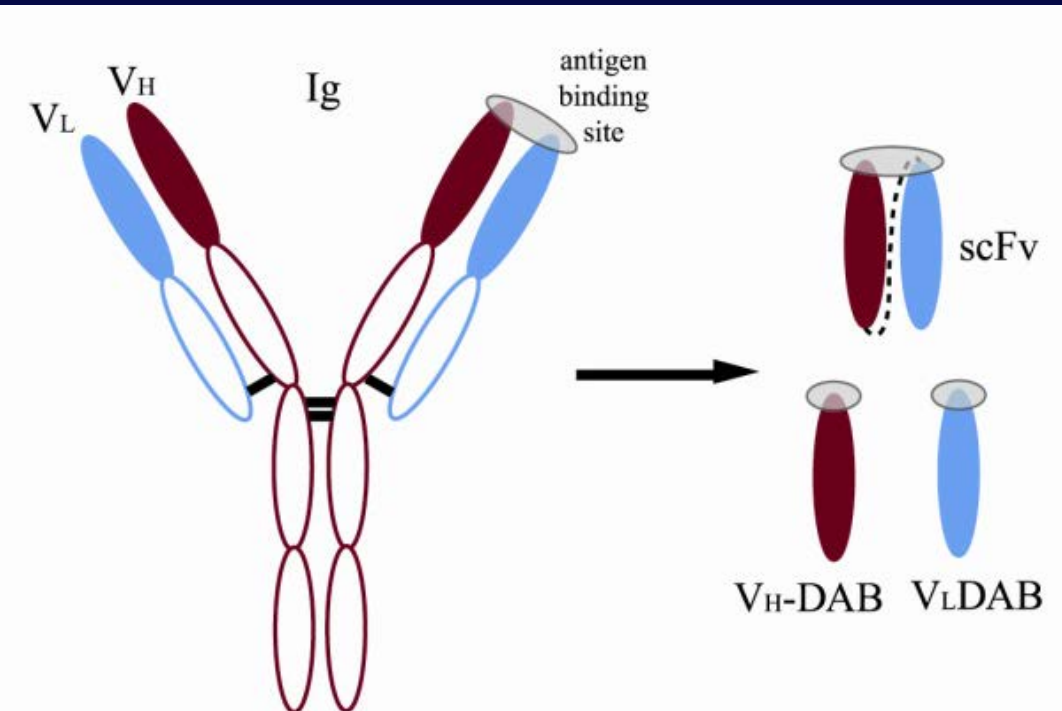
Two years

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

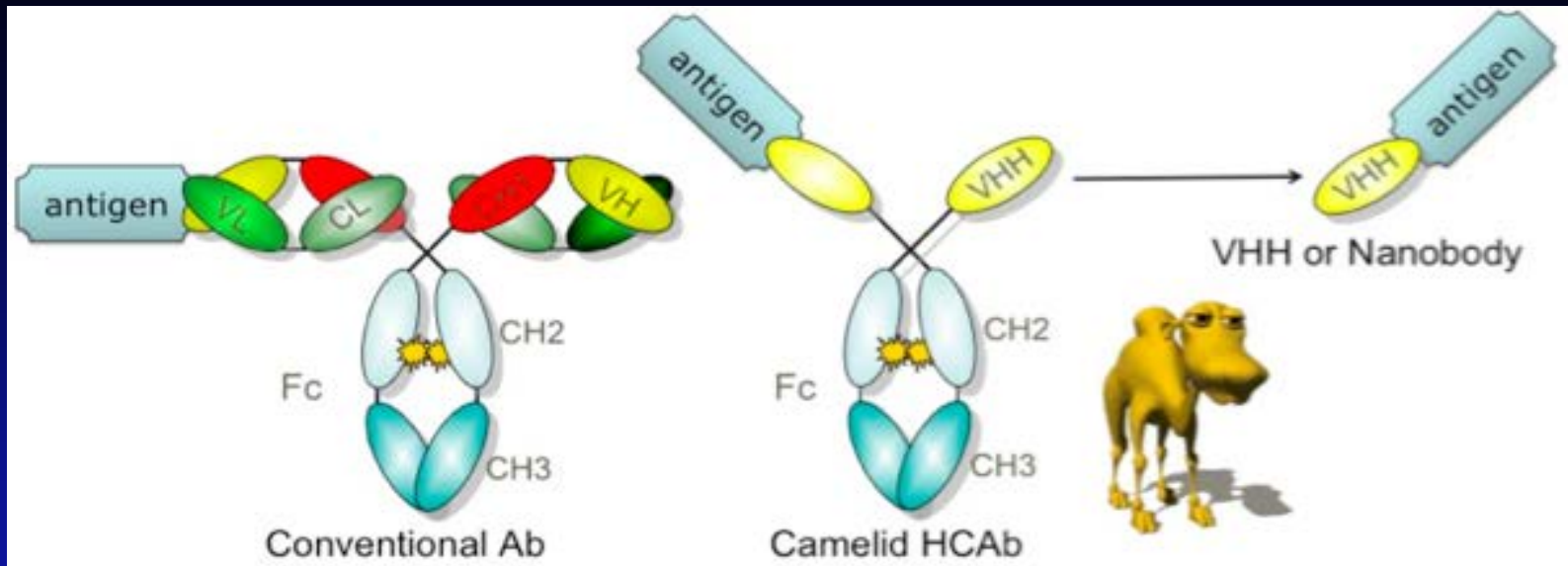


* ANCOVA model with a main effect for treatment group and baseline UPDRS Part III motor score in the practically defined off condition as covariate. Note: at 18 mos, 14 subjects have scores; therefore 16 subjects: LOCF

Intracellular antibodies (Intrabodies)



			<u>CDR1</u>	<u>CDR2</u>	<u>CDR3</u>															
A1	1	SGGGLVQPGGSLRLSCAVS	<u>GFSGFSLD</u>	YYPIAWFRQAPGKEREGV	<u>SCI-S-VAYNS</u>	VYTDSVKGRFTISRDN	AKNTVFLQMN	NLQPEDTAVYY	<u>CTA</u>	----	<u>PRPG</u>	----	<u>KTSCSSYVS</u>	<u>NPNWGSS</u>	W-----	GQGTQVT	VSS	125		
A3	1	TGGGLVQPGGSLRLSCAAS	<u>GFA</u>	---	<u>FRSST</u>	TSWYRQAPGKERELVAR	<u>LD-LTGLA</u>	TYADSVRGRFTVSRD	NVKNMLYLQMN	SLQPGDTATYY	<u>CKDI</u>	----	<u>PRGGGYES</u>	-----	<u>EYG</u>	----	GQGTQVT	VSS	115	
D5	1	SGGGLVQPGGSLRLSCAAS	<u>GS</u>	---	<u>FSFYR</u>	MAWYRQAPGKQREL	<u>VAYI-D-GPRS</u>	AVYARSVKGRFTISR	DDTKHMYVLQMN	SLQPEDTAVYY	<u>CNG</u>	-----	<u>QW</u>	-----	<u>IQSYW</u>	----	GQGTQVT	VSS	108	
E3	1	SGGGLVQPGGSLRLSCAAS	<u>GFT</u>	---	<u>FGSAG</u>	MSWFRQAPGKGLKW	<u>SSITS</u>	SRVADSVKGRFTISR	DNAKNTLYLQMN	SLKPDPTAVY	<u>CAKGF</u>	-----	<u>DS</u>	-----	<u>SWW</u>	PSRER	GQGTQVT	VSS	114	
E5	1	TGGGLVQPGGSLKLSCAVR	<u>PST</u>	---	<u>FNIDV</u>	VAWYRQAPGEQREL	<u>VAAI-T-GENNI</u>	DYAESVKGRFTISR	NAKKTAFLLMNS	LEPEDTAVYY	<u>CNADVFFPL</u>	<u>TRR</u>	-----	-----	<u>RVN</u>	YW----	GQGTQVT	VSS	115	
E9	1	TGGGLVRPGGSLRLSCTT	<u>SEII</u>	---	<u>QSISAMG</u>	WYRQAPGKERVLVAR	<u>DSPI</u>	<u>ISRGL</u>	TYADSVKGRFTISR	DNKNTLYLQMN	SLKPEDTAMYY	<u>CAT-F</u>	----	<u>PGWDSLVP</u>	-----	<u>DHW</u>	----	GQGTQVT	VSS	115
F1	1	SGGGLVQPGGTLRLSCVF	<u>SGS</u>	---	<u>FSFYG</u>	NWYRQAPGKERELVAT	<u>I-T-GPQFT</u>	KYADSVKGRFTVSR	DDAKNTVFLQMN	SLKPEDTAVYY	<u>CNA</u>	-----	<u>RW</u>	-----	<u>LTDY</u>	W----	GQGTQVT	VSS	108	
F5	1	TGGGLVQPGGSLRLSCAVS	<u>GFS</u>	---	<u>LDWYI</u>	IGWFRQAPGKEREGV	<u>SCI-T-PACOT</u>	IYADSVKDRFTISR	DNAKNTAFQLNL	NLKPEDTAVYY	<u>CTT</u>	----	<u>PRPG</u>	----	<u>KTSCSDYVS</u>	<u>NPNWGSS</u>	W-----	GQGTQVT	VSS	122
F12	1	SGGGLVQPGGSLRLSCAAS	<u>GRS</u>	---	<u>MDVYI</u>	IGWFRQAPEKEREGV	<u>SCI-G-AAWNI</u>	YADSVKGRFTISR	DNA--	AVYLMNNL	KPEDTGTY	<u>YCAA</u>	----	<u>KTHHWRET</u>	<u>LORMCAMTPD</u>	<u>IYW</u>	----	GQGTQVT	VSS	122

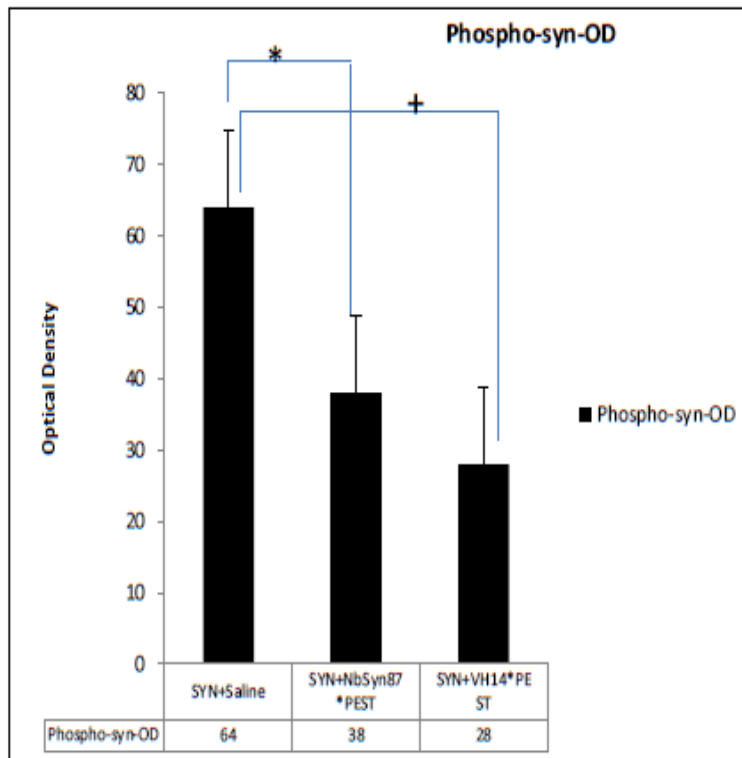


- Able to recognize antigenic sites inaccessible to conventional antibodies.
- V_H Hs have a high tolerance to changes in temperature and pH.
- Minimally immunogenic
 - Framework similar to that of human V_H family 3
 - Can be humanized

V_H H nanobodies

C. Substantia Nigra

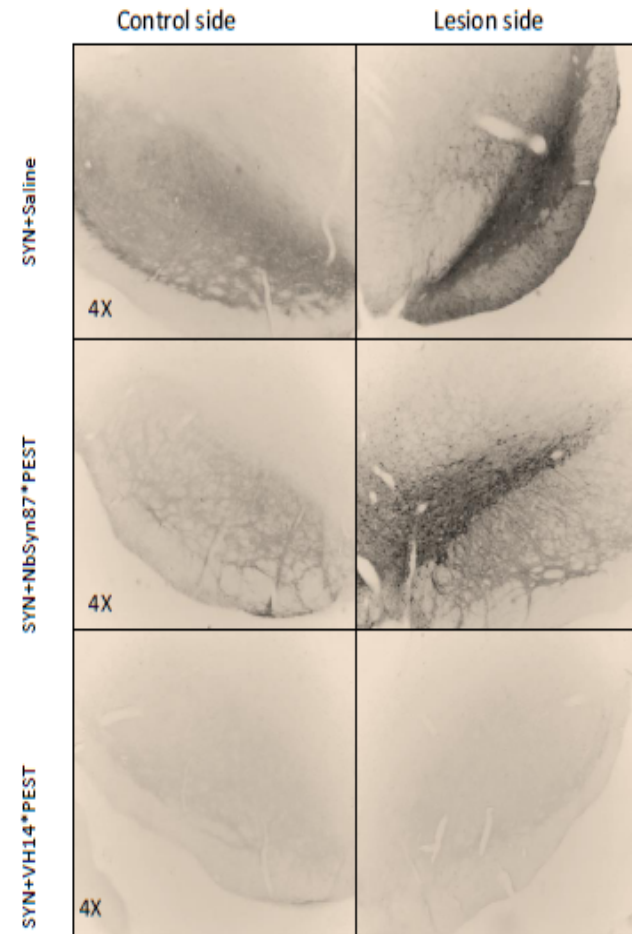
4. phospho-synuclein



ANOVA yields a significance with $F(2,20)=4.816$ $p=0.021$

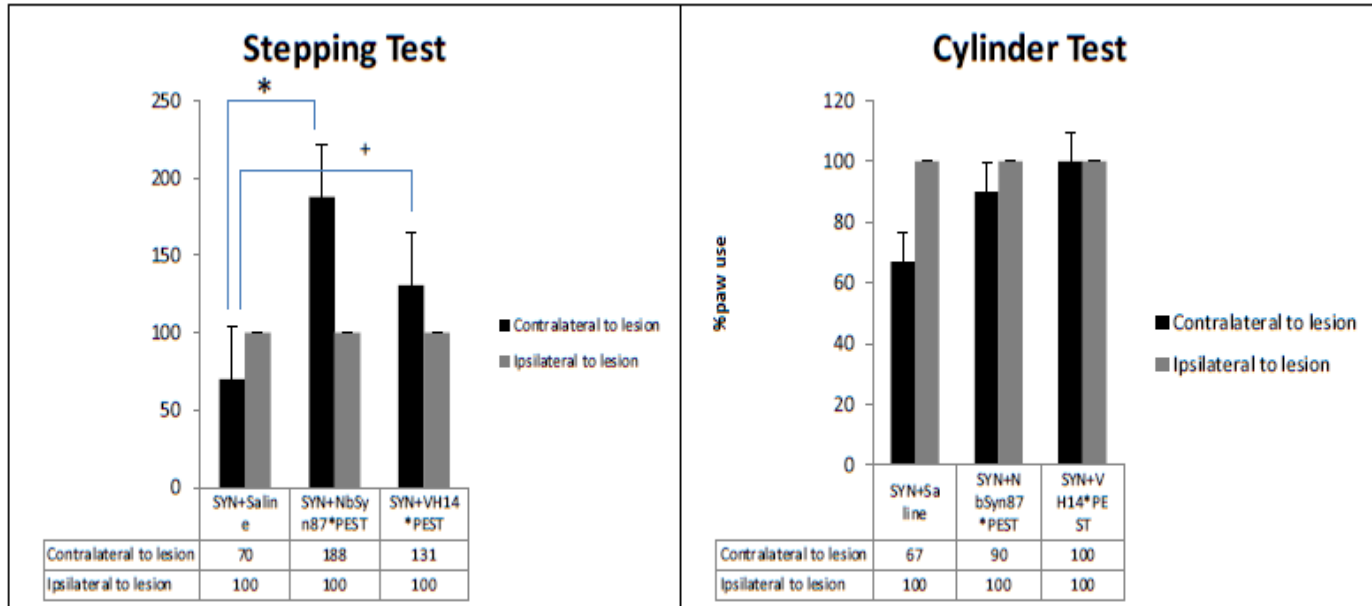
* $p=0.044$ (LSD post-hoc)

+ $p=0.021$ (Bonferroni post-hoc)



Representative photographs of animals from each group stained for phosphorylated α -syn (pathological form of α -synuclein) demonstrated none in VH14*PEST treated group while similar levels of phospho-syn in Saline and NbSyn87*PEST groups.

A. Behavioral Analysis



A two-way ANOVA yields a significant interaction with $F(2,80)=3.260$, $p=0.044$.

* $p=0.001$ (Bonferroni post-hoc) significantly higher than Saline treated

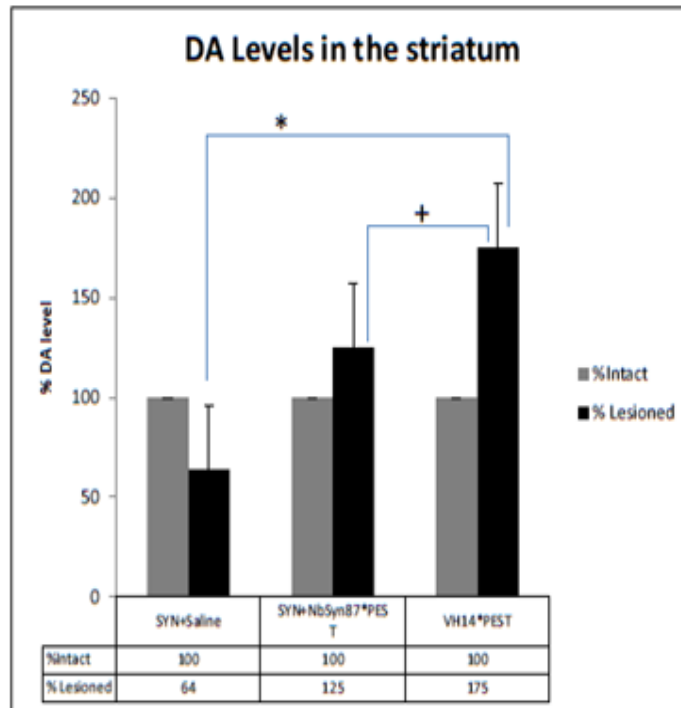
+ $p=0.055$ (LSD post-hoc) significantly higher than Saline treated

Note: Contralateral side values are to be noted

Note: Contralateral side values are to be noted

B. Striatum

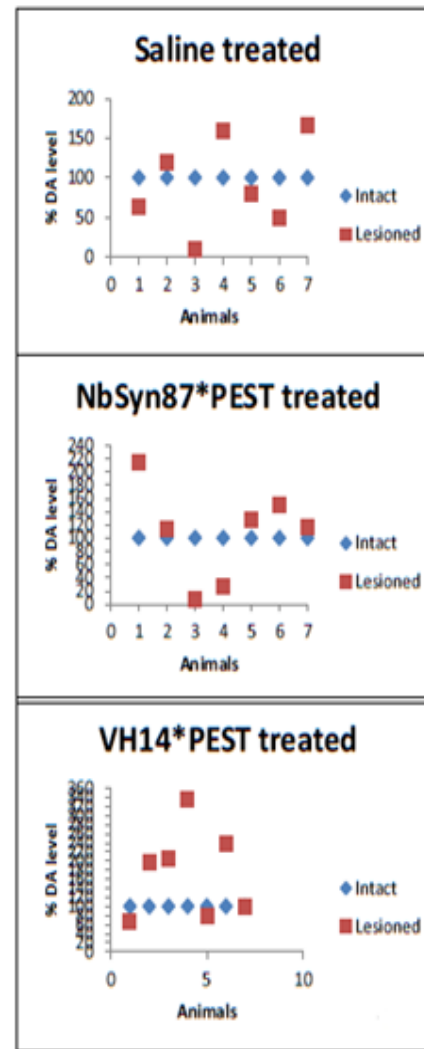
4. Dopamine levels as determined by HPLC



A two-way ANOVA yields a significant interaction with $F(2,32)=3.121$ $p=0.05$

* $p=0.005$ (Bonferroni post-hoc)

+ $p=0.002$ (LSD post-hoc)



Conclusions

- Understand the status of the human disease at a time of the clinical course based upon the inclusion criteria
- Model preclinically that clinical entity and appreciate to limitations of the models even more than appreciating the models similarities to the human disease.
- Interpret your data with rigor and honesty. It will save you (a lot of) time and (a lot of) money and it will not compromise the safety of the brave patients that enter these trials

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As well as all clinical site neurologists, neurosurgeons, coordinators, and patients