DBS Plus: Peripheral Nerve Grafting Combined with DBS to Possibly Slow or Modify the Progression of Parkinson's Disease

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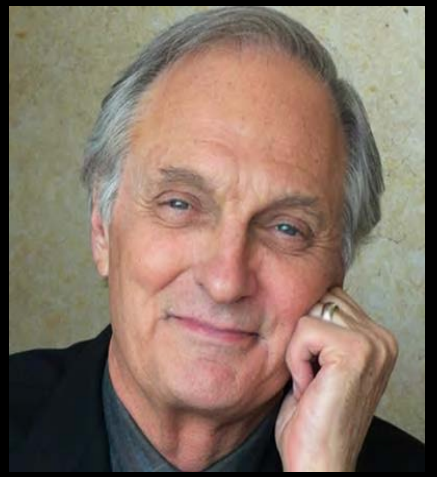
Disclosures

Off-label use of DBS using reverse staging approach (van Horne et al., 2015) and implantation of DBS leads into GPi under anesthesia.

Greg Gerhardt is a founder of Avast Pharmaceuticals, LLC and has two US patents licensed from the University of Kentucky.

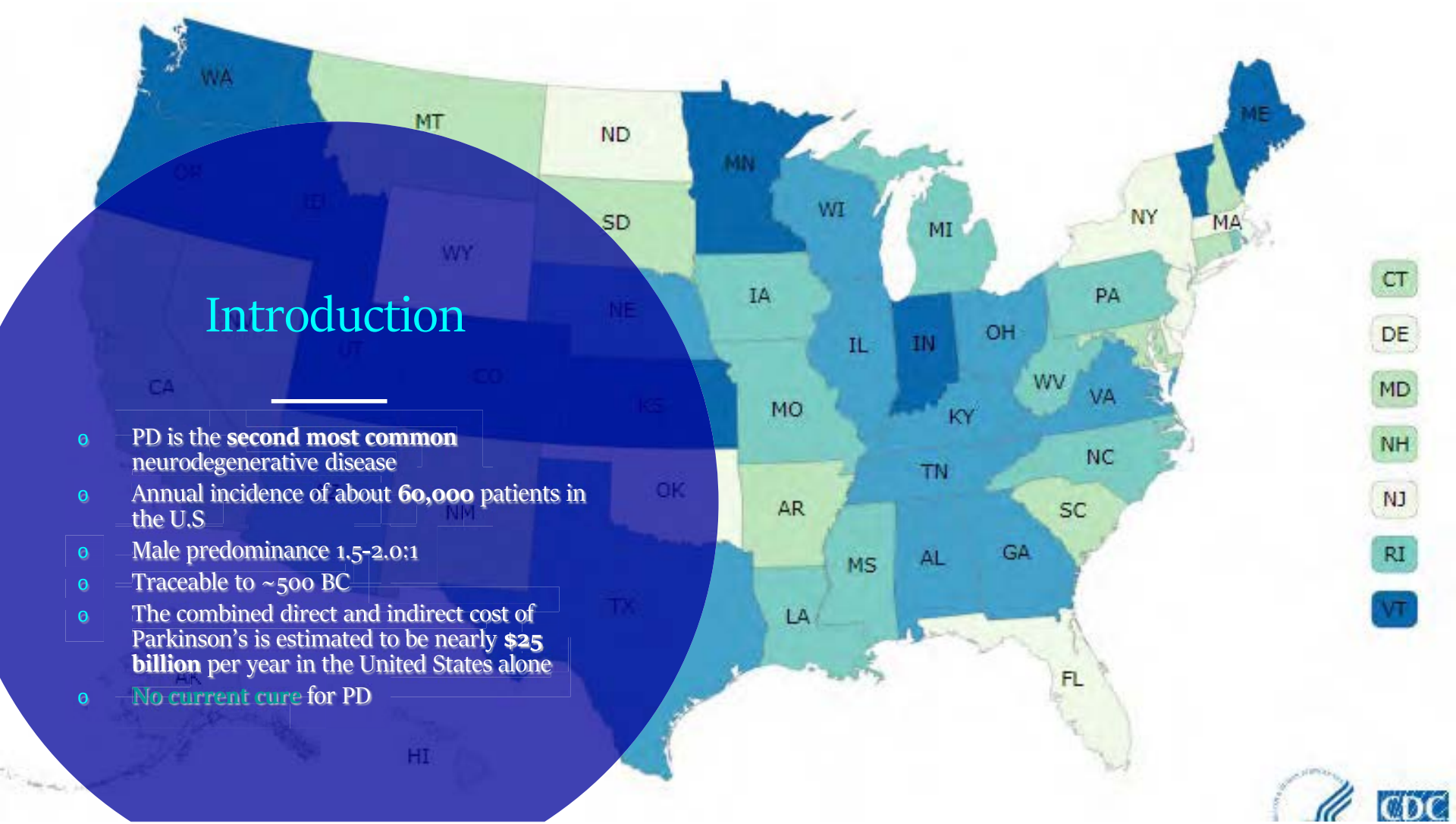
Two ongoing Phase I clinical trials (discussed with FDA): Clinicaltrials.gov: NCT01833364, NCT02369003

NOTABLE PEOPLE WITH PARKINSON'S SYNDROME/ SPECTRUM



Introduction

- PD is the **second most common** neurodegenerative disease
- Annual incidence of about **60,000** patients in the U.S
- Male predominance 1.5-2.0:1
- Traceable to ~500 BC
- The combined direct and indirect cost of Parkinson's is estimated to be nearly **$25 billion** per year in the United States alone
- No current cure for PD



MOTOR & NON-MOTOR SYMPTOMS

Motor Symptoms (2 of 4):

- Bradykinesia / Shuffling Gait
- Rigidity (cogwheel or lead-pipe)
- Resting Tremor (70% of patients)
- Stooped Posture / Unstable Balance

Parkinson's disease

Source: www.idiom.com

Non-Motor Symptoms:

- Dysphagia (difficulty swallowing)
- Depression (>40% early sign)
- REM Sleep Disorder (>40% of patients)
- Cognitive changes (memory)
- Loss of the sense of smell
- GI tract dysfunction (constipation)
- Fatigue

Diagnosis of PD remains clinical

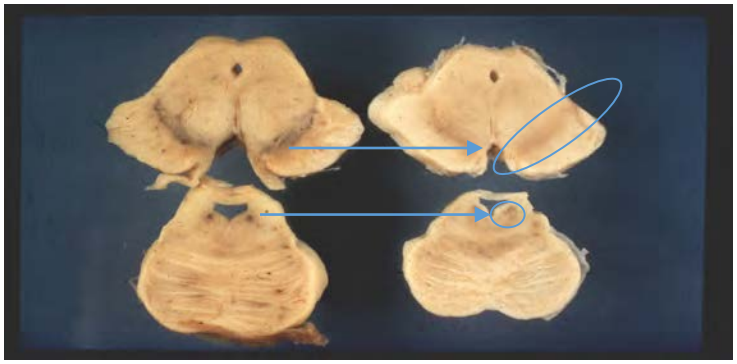
Neurons Affected in PD: Not *just* a Disease of DA Neurons

1. Dopamine neurons in SN and VTA (Zarow et al., 2003; Uhl et al., Neurology, 1985)
2. Noradrenergic Neurons in Locus Coeruleus (Zarow et al., JAMA, 2003)
3. Serotonin Neurons in Dorsal/Median Raphe (Halladay et al, Brain Res. 1990; Lang et al., Neuroplogy, 2007)
4. Cholinergic Neurons in Nucleus Basalis of Meynert (Liu et al., Acta Neuropathol., 2015; Zarow ert al., JAMA, 2003)
5. Substance P Neurons (Halladay et al, Brain Res. 1990)

Idiopathic Parkinson's Disease

Pathology and Biochemistry

- **Pathologically:** characterized by the degeneration of dopamine neurons in the substantia nigra and noradrenergic neurons in the locus coeruleus, which are pigmented areas located in the brainstem. (C Tretiakoff, 1919).



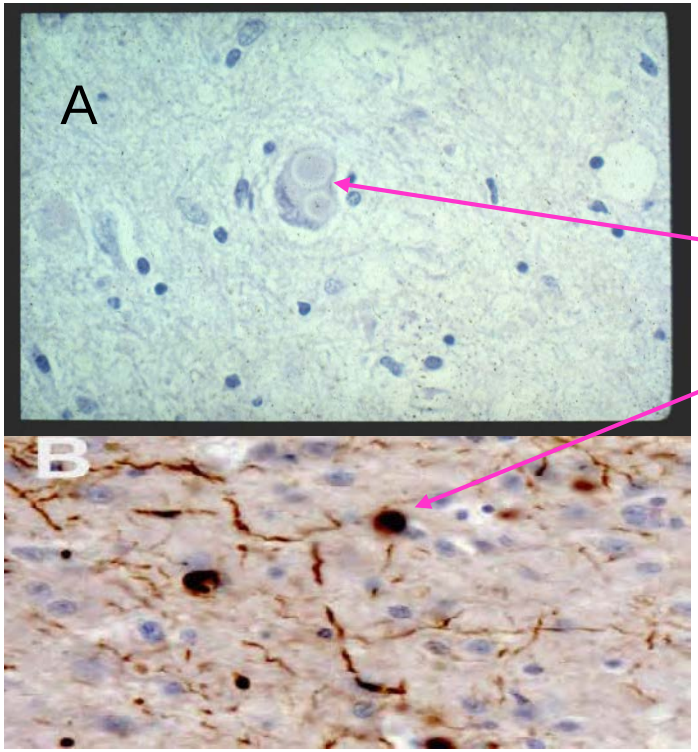
- **Biochemically:** characterized by >80% depletion of the neurotransmitter dopamine in the striatum (Arvid Carlsson, 1959). Provides rationale for replacement therapy with dopaminergic drugs.

- Losses of other neuron groups: Ventral Tegmental Area, Locus Coeruleus, Dorsal Raphe, Nucleus Basalis of Meynert.

- Braak and Gerhardt conversation 2008: "Neuronal loss in PD is selective for poorly myelinated neurons".

Idiopathic Parkinson's Disease

Histopathology



The Lewy body

- Intracytoplasmic hyaline inclusion
  - α - synuclein in core
  - Ubiquitin at rim

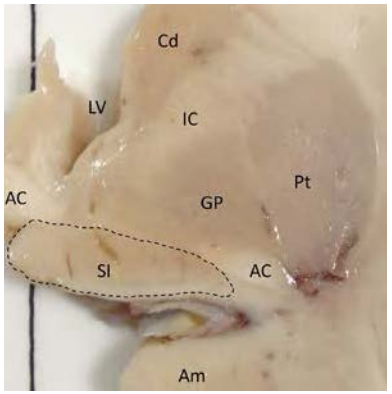
Is α - synuclein a prion?

Lewandowsky's Handbook of Neurology, 1912; German Association of Psychiatrists and Neurologists, Breslau, 1913

Chu Y, Kordower JH. The prion hypothesis of Parkinson's disease. Current Neurol & Neurosci Report, 2015; 15(5):28. doi: 10.1007/s11910-015-0549-x.

Nucleus basalis of Meynert (nbM)

- Cholinergic rich region of the basal forebrain
- Projects primarily to the cortex as well as the amygdala
- Lewy bodies and neuronal loss first identified in nbM (loss in PDD is greater than AD)
- Nerve growth factor (NGF) provides support



From Lun Liu (2015)

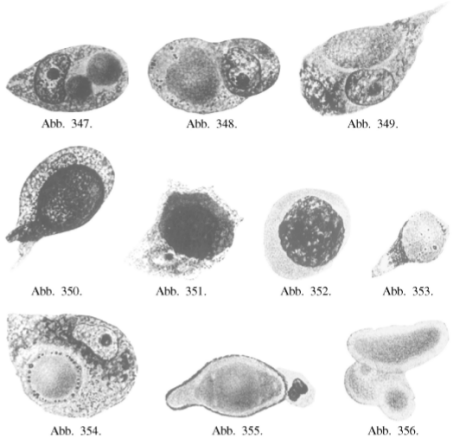
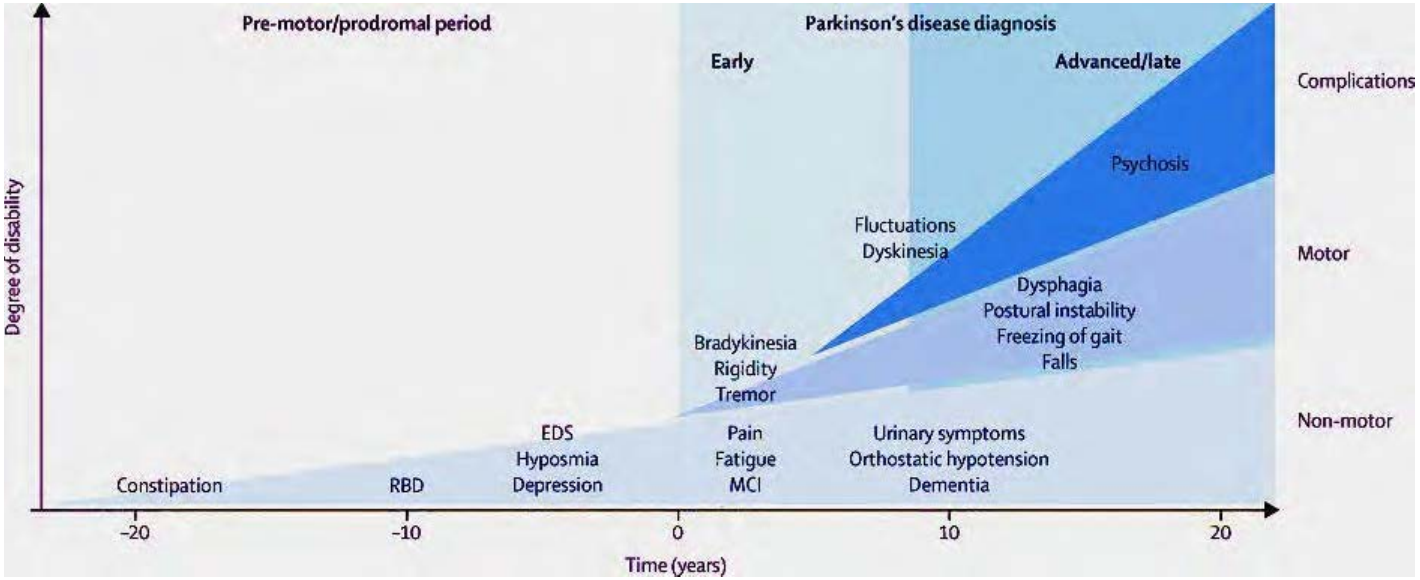


Fig. 3. Lewy 1923: Inclusion bodies from the nucleus basalis (Bielschowsky silver staining): drop-like alterations, inclusion bodies and cell degeneration. Lower part: inclusion bodies surrounded by argentophilic granula.

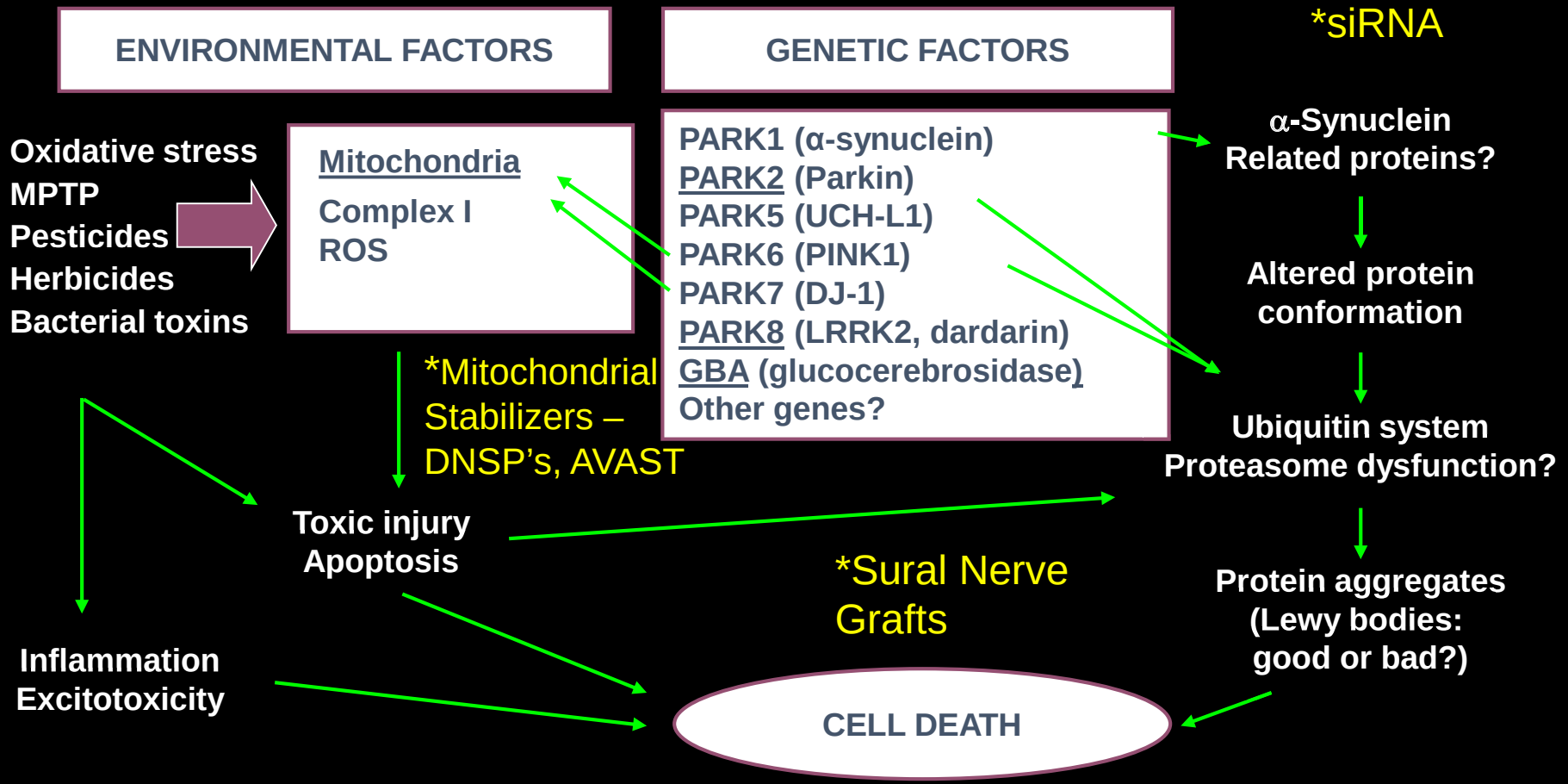
From Holdorff (2002)

Next Big Step in Treatment of Neurodegenerative Diseases

"*Disease-modifying treatments* that reduce the rate of neurodegeneration or stop the disease process have remained elusive and are the greatest unmet therapeutic need in Parkinson's disease." (Lane, 2016).



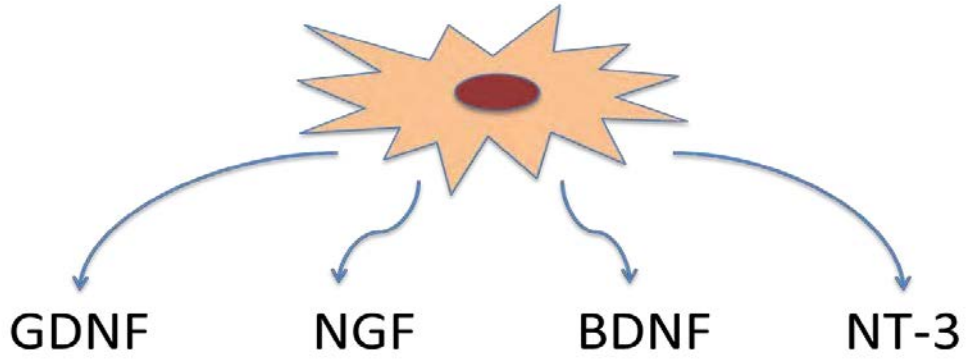
Potential Mechanisms and Targets of Neurodegeneration



BenMoyal-Segal L, Soreq H. *J Neurochem*. 2006;97:1740-1755.
Dawson TM, Dawson VL. *J Clin Invest.* 2003;111:145-151.
Mouradian MM. *Neurology*. 2002;58:179-185.

DBS Plus: Combined Cell Therapy, DBS and Pharmacotherapy

Delivery of "repair Schwann cells" - transdifferentiated Schwann cells from a Patient's own sural nerve



Clinicaltrials.gov: NCT01833364, NCT02369003

Why Peripheral Nerve Tissue?

- ✓ The PNS, in contrast to the CNS, promotes axonal regeneration following injury.
- ✓ The PNS environment supports axonal growth and elongation for CNS axons over long distances
- ✓ "Cocktail of Trophic Factors, Anti-apoptotic factors, Other Pro-repair factors"

(Albert Aguayo 1980s)

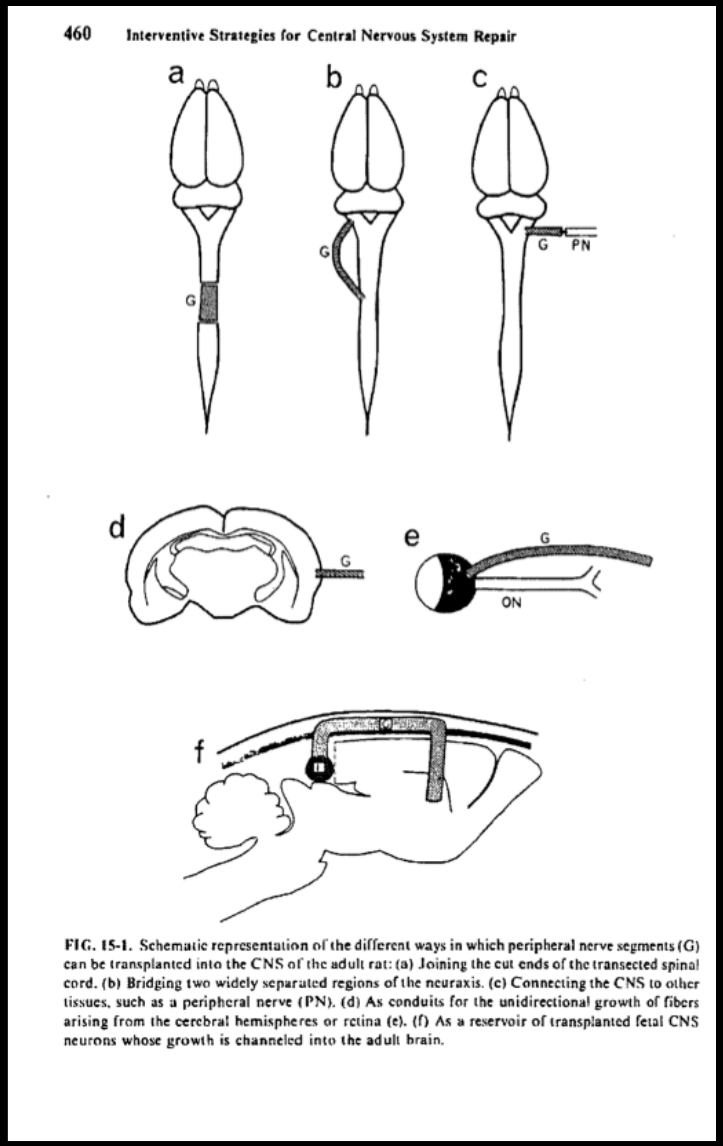
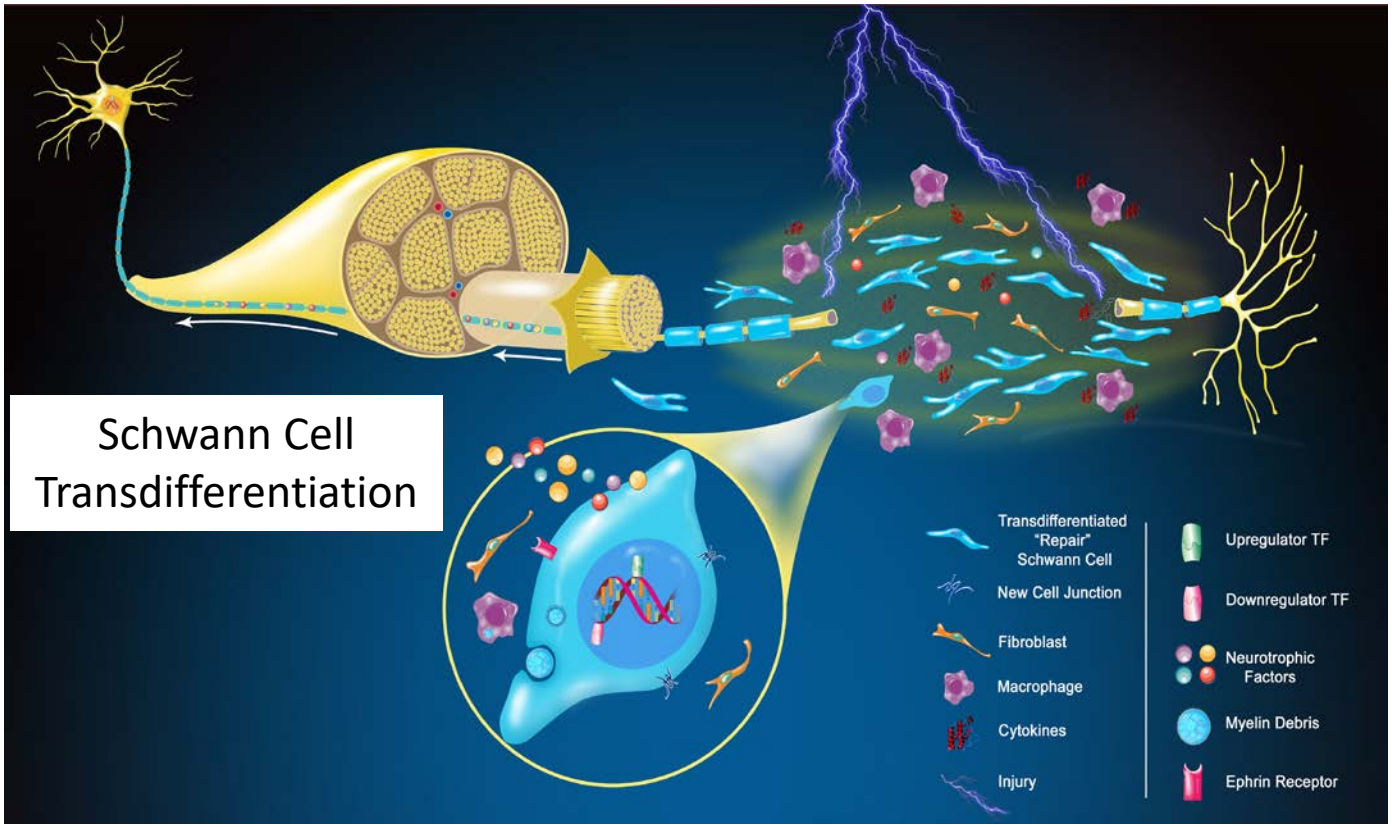
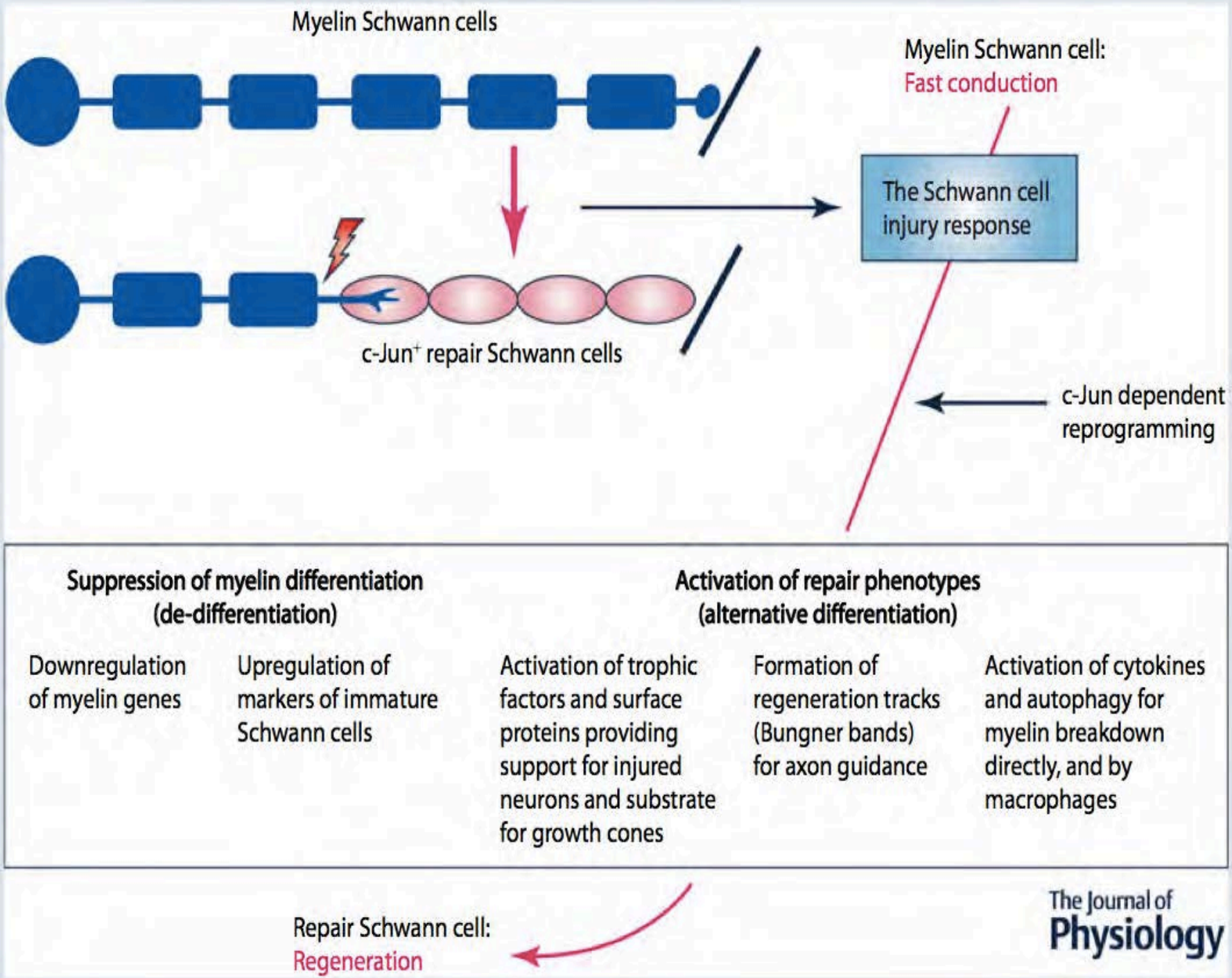


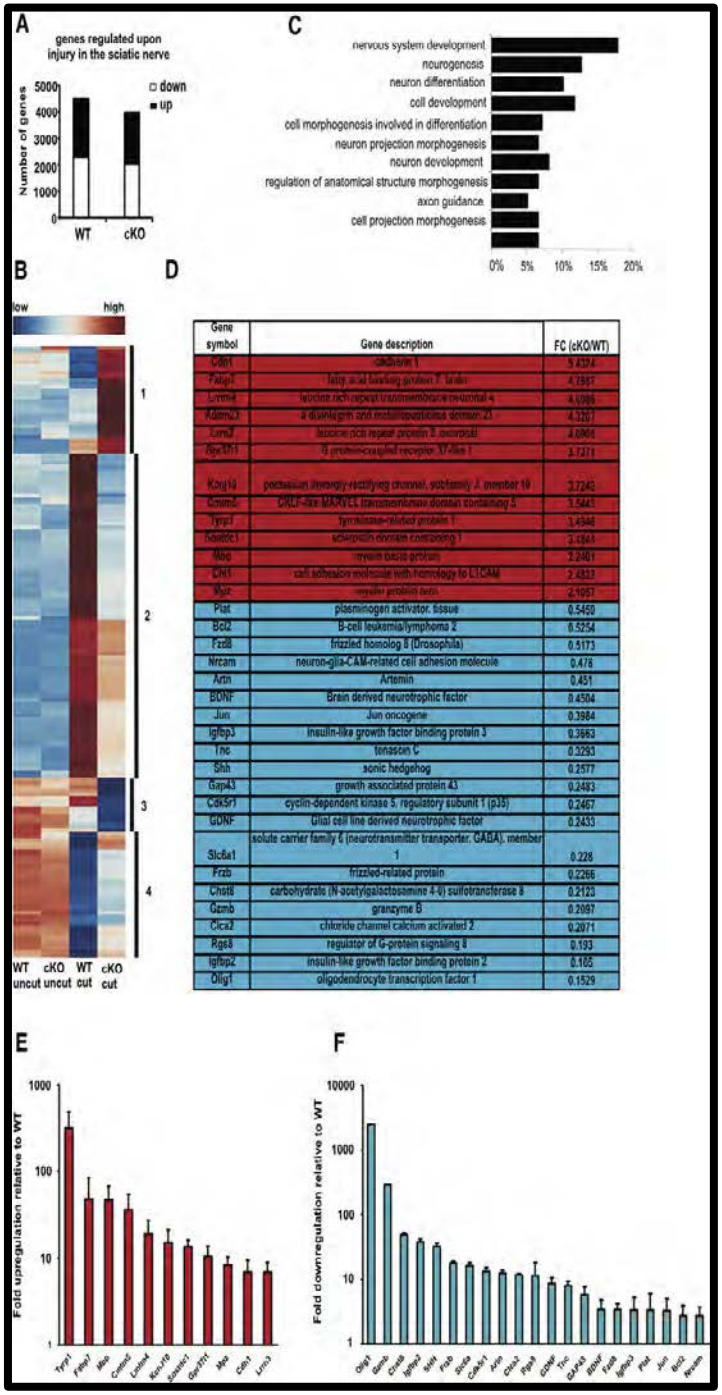
FIG. 15-1. Schematic representation of the different ways in which peripheral nerve segments (G) can be transplanted into the CNS of the adult rat: (a) Joining the cut ends of the transected spinal cord. (b) Bridging two widely separated regions of the neuraxis. (c) Connecting the CNS to other tissues, such as a peripheral nerve (PN). (d) As conduits for the unidirectional growth of fibers arising from the cerebral hemispheres or retina (e). (f) As a reservoir of transplanted fetal CNS neurons whose growth is channeled into the adult brain.

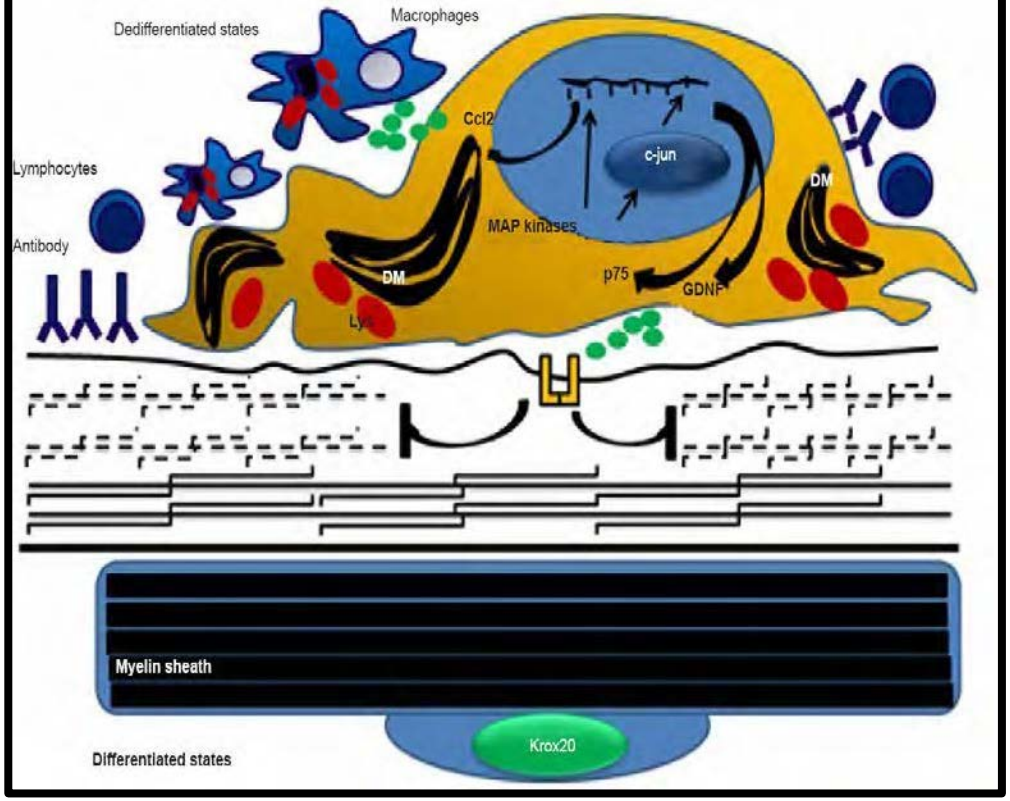
Peripheral nerves can regenerate after injury and re-establish function

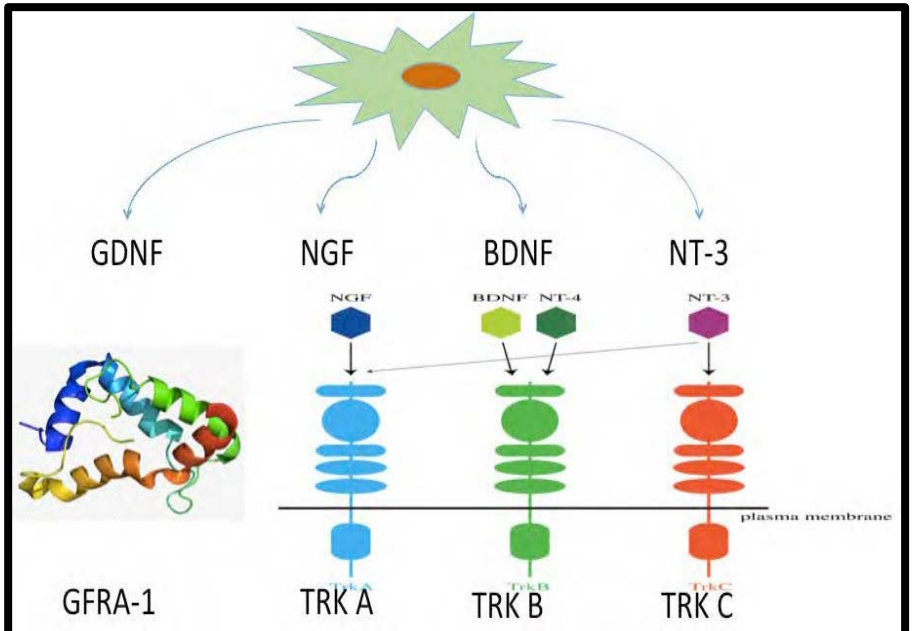




The Journal of Physiology


A
genes regulated upon injury in the sciatic nerve
Number of genes
down
up
WT
cKO
C
nervous system development
neurogenesis
neuron differentiation
cell development
cell morphogenesis involved in differentiation
neuron projection morphogenesis
neuron development
regulation of anatomical structure morphogenesis
axon guidance
cell projection morphogenesis
0%
5%
10%
15%
20%
B
low
high
WT uncut
cKO uncut
WT cut
cKO cut
D
Gene symbol
Gene description
FC (cKO/WT)
Plat
plasminogen activator, tissue
0.5459
Bcl2
B-cell leukemia/lymphoma 2
0.5254
Fzd8
frizzled homolog 8 (Drosophila)
0.5173
Nrcam
neuron-glia-CAM-related cell adhesion molecule
0.478
Artn
Artemin
0.451
BDNF
Brain derived neurotrophic factor
0.4504
Jun
Jun oncogene
0.3984
Igfbp3
insulin-like growth factor binding protein 3
0.3663
Tnc
tenascin C
0.3293
Shh
sonic hedgehog
0.2577
Gap43
growth associated protein 43
0.2483
Cdk5r1
cyclin-dependent kinase 5, regulatory subunit 1 (p35)
0.2467
GDNF
Glial cell line derived neurotrophic factor
0.2433
Slc6a1
solute carrier family 6 (neurotransmitter transporter, GABA), member 1
0.228
Frzb
frizzled-related protein
0.2266
Chst8
carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 8
0.2123
Gzmb
granzyme B
0.2097
Clca2
chloride channel calcium activated 2
0.2071
Rgs8
regulator of G-protein signaling 8
0.193
Igfbp2
insulin-like growth factor binding protein 2
0.182
Olig1
oligodendrocyte transcription factor 1
0.1529
E
Fold upregulation relative to WT
F
Fold downregulation relative to WT

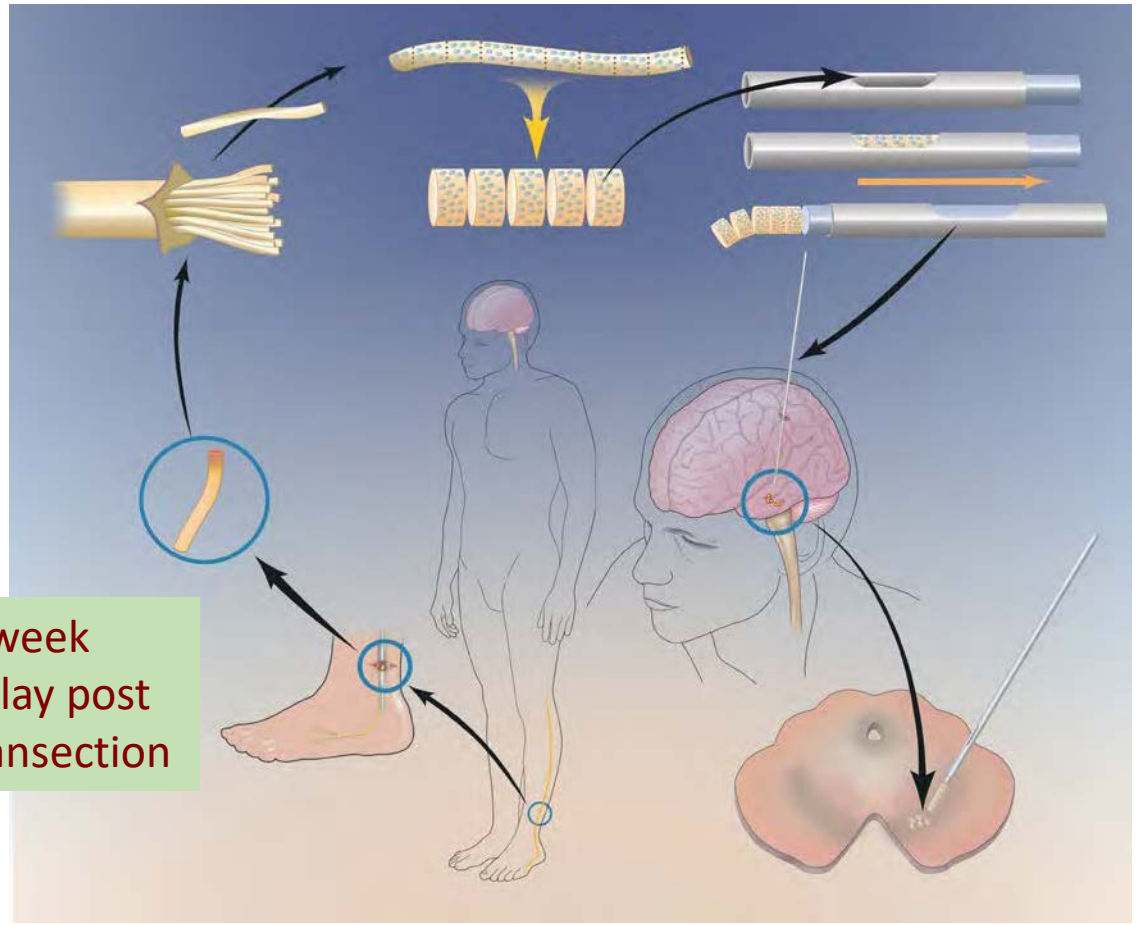

Dedifferentiated states
Macrophages
Ccl2
c-jun
Lymphocytes
DM
MAP kinases
Antibody
DM
p75
GDNF
Myelin sheath
Krox20
Differentiated states


GDNF
NGF
BDNF
NT-3
NGF
BDNF
NT-4
NT-3
plasma membrane
GFRA-1
TRK A
TRK B
TRK C

Sural Nerve Implants from a Practical Standpoint:

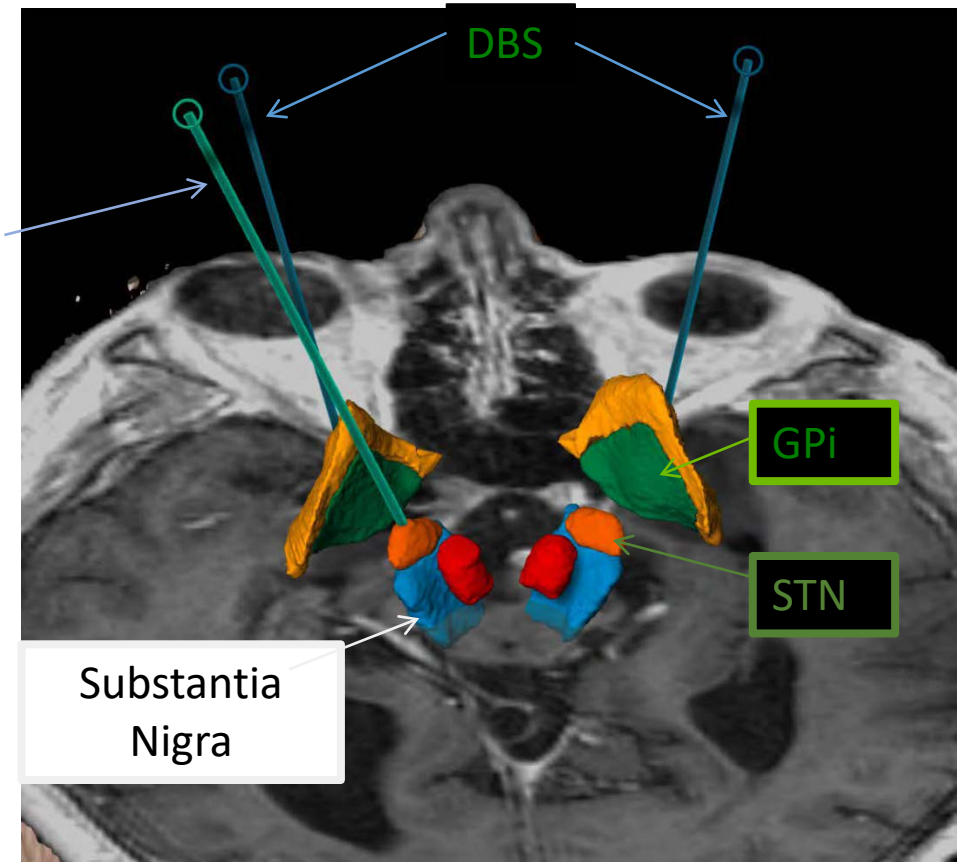
1. Participants supply their own tissue.
2. PNS tissue is abundant and can be obtained with minimal risk.
3. For the purposes of this study, no modifications to the tissue are required.
4. Participants receive full benefit of DBS therapy
5. The procedure can be performed at the time of DBS surgery, adding only 30-45 minutes of operative time.

Overview of Nerve Graft Harvesting and DBS+ Implant



DBS *Plus*

Graft cannula



Study Details

- Exploratory Phase I clinical trials (n=70 to date) with primary outcomes focused on *feasibility and safety*
- Open label, non-blinded, non-randomized
- Investigator initiated (no corporate sponsor) with internal and philanthropy funding
- No conflicts of interest related to this work
- Clinical Trials NCT01833364, NCT02369003 (ClinicalTrials.Gov)

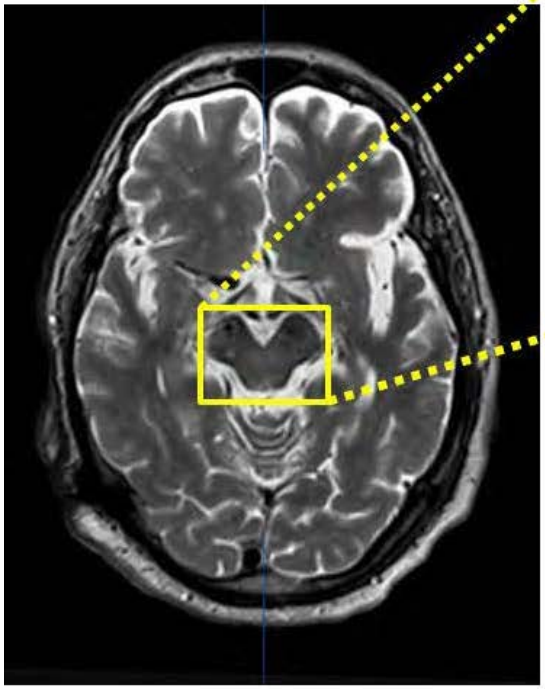
Selection Criteria for DBS in PD in US Patients (Only about 10-20% qualify)

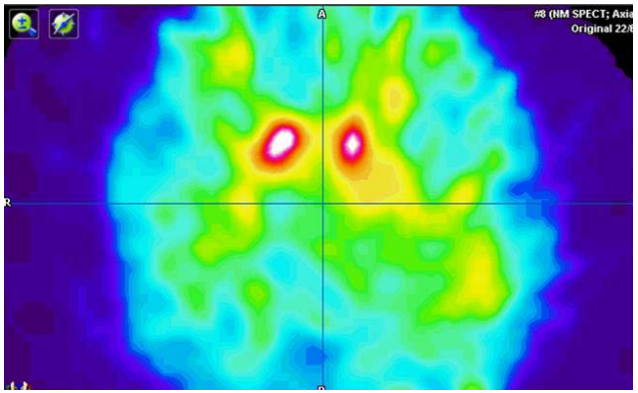
- PD > 5 years (to rule out atypical cases)
- Levodopa responsiveness
- Troublesome motor fluctuations
- Troublesome dyskinesia
- Disabling refractory tremor
- Optimal medical management
- Medication intolerance
- Normal MRI
- Exclude atypical and secondary parkinsonism
- Exclude dementia and depression
- Good medical health
- Realistic expectations

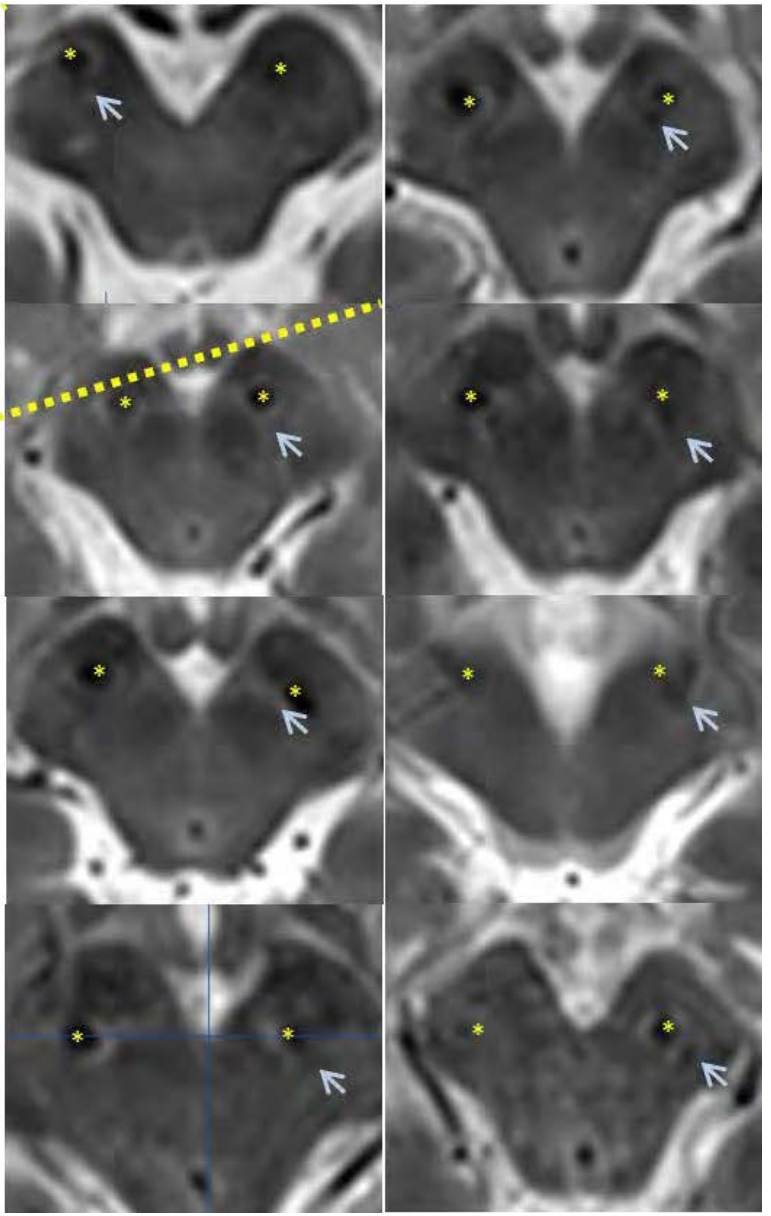
Incidence of Adverse Events

MedDRA Primary System Organ Classification	Number of Participants Experiencing (Affected/Total)	AE Rated Possibly, Probably, Definitely Related to Study Intervention (Affected/Total)
Cardiac disorders	1/18	——
Endocrine disorders	1/18	——
Eye disorders	2/18	——
Gastrointestinal disorders	2/18	——
General disorders and administration site conditions (e.g. fatigue)	1/18	——
Infections and infestations (e.g. UTI, device related infection, wound infection, sepsis, etc.)	7/18	——
Ankle wound site infection		**1/18**
Injury, poisoning and procedural complications (e.g. falls, fractures, wound complications)	5/18	——
Investigations (e.g. weight loss)	2/18	——
Metabolism and nutrition disorders	1/18	——
Musculoskeletal and connective tissue disorders	5/18	——
Nervous system disorders	6/18	——
Paresthesia of foot/ankle		**5/18**
Psychiatric disorders	3/18	——
Respiratory, thoracic and mediastinal disorders	2/18	——
Vascular disorders	1/18	——

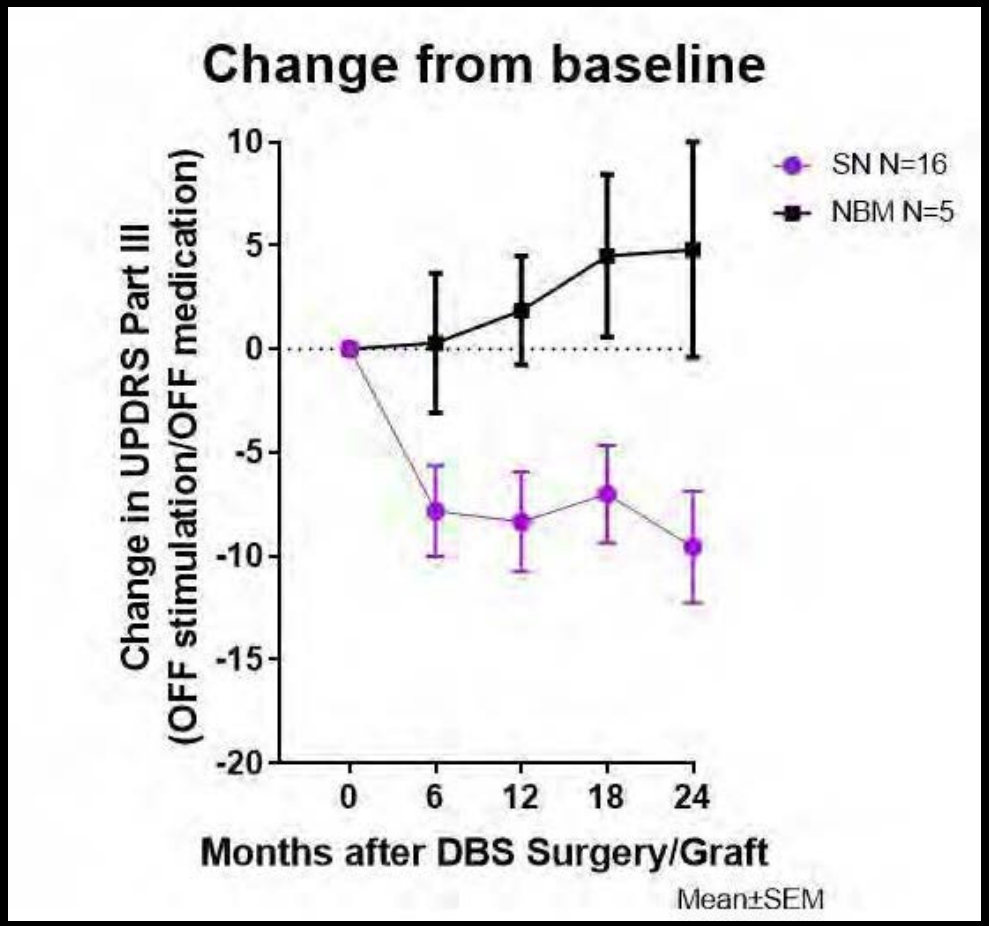
12 Month MRI

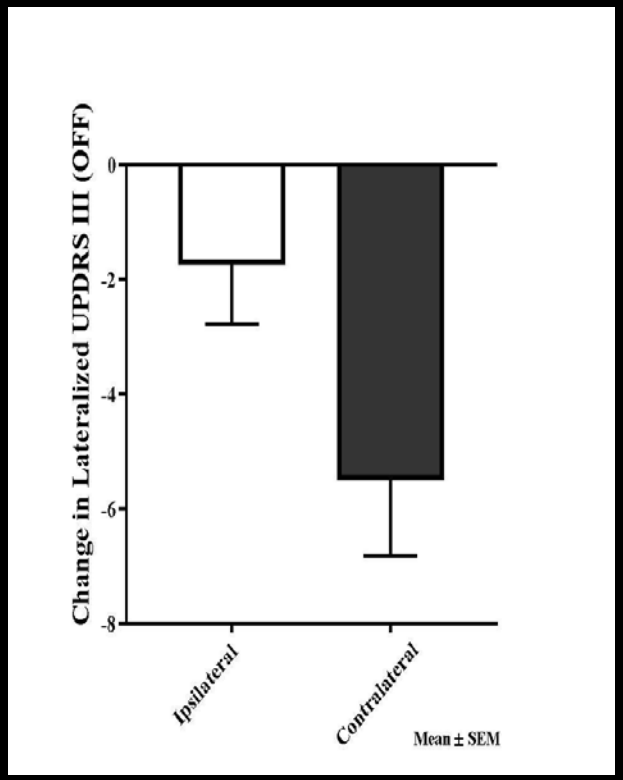






UPDRS Part II Motor Scores OFF stim/ OFF med. for 21 Subjects at 2 Years

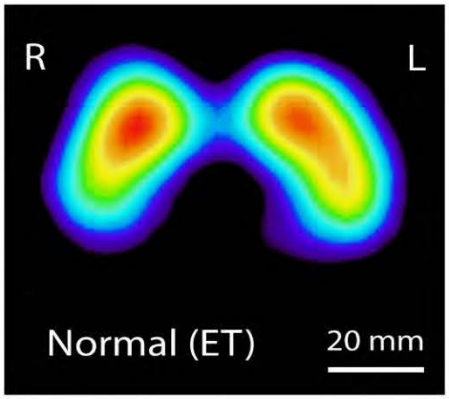


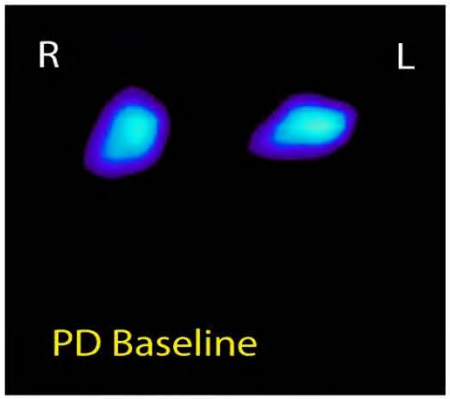


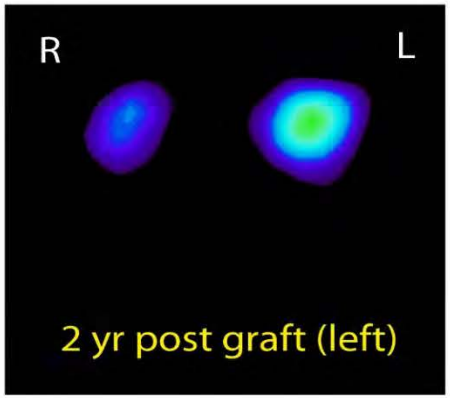
How do Peripheral Nerve Grafts Work?: Dopamine transporter single photon emission (DaT-SPECT)

- (^{123}I)FP-CIT or DaTSCAN or DaTscan

- FDA approved in 2011 in US “to help differentiate ET from tremor due to parkinsonian syndromes”





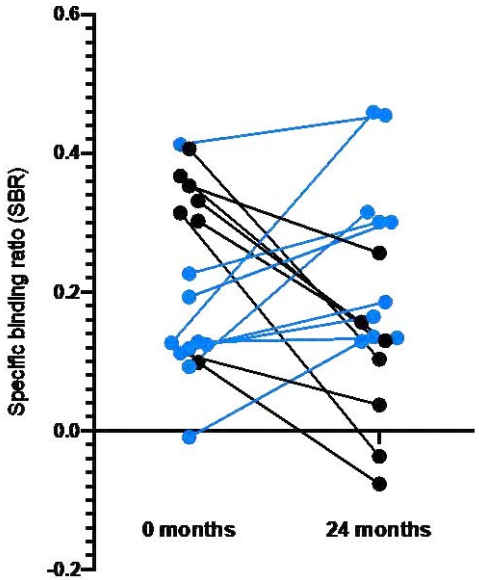


DAT Quant analysis (0 & 24mo after implant)

Posterior putamen

Ipsilateral injected side shown

Blue= increased SBR after 24 months



0 months	24 months
0.3672	0.1353
0.0985	-0.0765
0.4125	0.4547
0.314	-0.037
0.3024	0.1567
0.0925	0.3153
-0.0093	0.1288
0.1279	0.1337
0.4066	0.103
0.2262	0.3008
0.1268	0.4593
0.1121	0.0373
0.1929	0.3009
0.3529	0.2563
0.1239	0.1646
0.3313	0.1298
0.1187	0.1856

Increased DAT = improved UPDRS?

UPDRS III motor		Decreased?	DAT	increased?	
	OFF	OFF	Ipsi posterior putamen		Compare: DAT and UPDRS III
participant	Baseline	24 months	0 months	24 months	
1	29	38	0.3672	0.1353	
2	25	14	0.0985	-0.0765	
4	48	29	0.4125	0.4547	increased in DAT/ decreased UPDRS III
5	35	30	0.314	-0.037	
7	23	19	0.3024	0.1567	
8	51	51	0.0925	0.3153	
9	46	37	-0.0093	0.1288	increased in DAT/ decreased UPDRS III
10	47	28	0.1279	0.1337	increased in DAT/ decreased UPDRS III
12	20	19	0.4066	0.103	
13	39	13	0.2262	0.3008	increased in DAT/ decreased UPDRS III
18	22	24	0.1268	0.4593	
19	37	20	0.1121	0.0373	
21	54	24	0.1929	0.3009	increased in DAT/ decreased UPDRS III
22	48	32	0.3529	0.2563	
24	38	36	0.1239	0.1646	increased in DAT/ decreased UPDRS III
25	40	35	0.3313	0.1298	
28	56	51	0.1187	0.1856	increased in DAT/ decreased UPDRS III
					7/17 participants

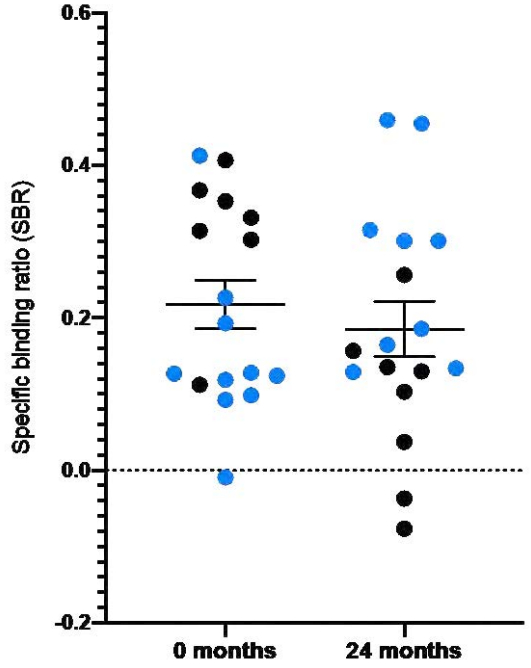
DAT Quant analysis
(0 & 24mo after implant)

Posterior putamen

Ipsilateral injected side shown

Blue= increased SBR after 24 months

Composite- Mean +/- SEM



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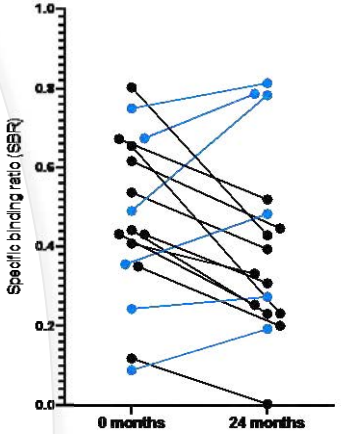
0.314	-0.037
0.3024	0.1567
0.0925	0.3153
-0.0093	0.1288
0.1279	0.1337
0.4066	0.103
0.2262	0.3008
0.1268	0.4593
0.1121	0.0373
0.1929	0.3009
0.3529	0.2563
0.1239	0.1646
0.3313	0.1298
0.1187	0.1856

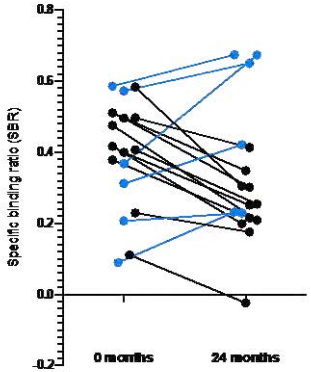
DAT in other areas

Anterior Putamen
Ipsilateral injected side shown
Blue= increased SBR after 24 months



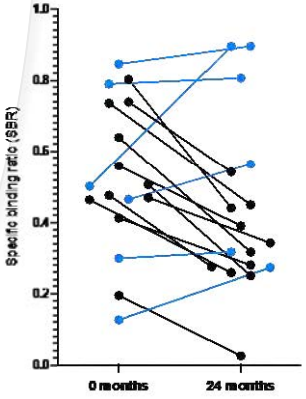
0 months	24 months
0.4422	0.2313
0.1182	0.0031
0.6741	0.7864
0.4083	0.3325
0.6166	0.4464
0.0885	0.1929
0.3508	0.2003
0.2438	0.2738
0.5376	0.3939
0.356	0.4832
0.4908	0.7831
0.8026	0.4292
0.7493	0.8134
0.4322	0.2537
0.6545	0.2321
0.4315	0.3082
0.6727	0.5197

Total putamen
Ipsilateral injected side shown
Blue= increased SBR after 24 months



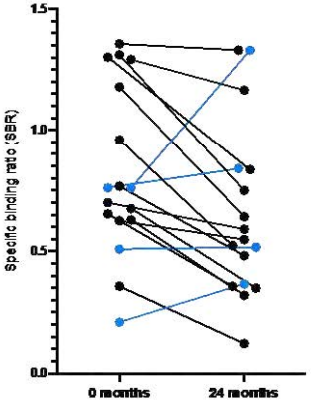
0 months	24 months
0.4169	0.1989
0.1116	-0.0238
0.5859	0.6745
0.3784	0.2152
0.5106	0.3487
0.0898	0.2318
0.2294	0.1762
0.207	0.2294
0.496	0.3016
0.3122	0.4217
0.5881	0.6739
0.5835	0.3048
0.5727	0.6508
0.407	0.2545
0.4756	0.2094
0.3997	0.2516
0.4869	0.4137

Total Striatum
Ipsilateral injected side shown
Blue= increased SBR after 24 months

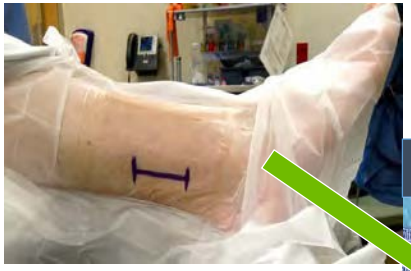


0 months	24 months
0.5039	0.2496
0.1942	0.0254
0.8447	0.8949
0.4612	0.2578
0.7351	0.4473
0.1259	0.2724
0.4102	0.2792
0.2978	0.3158
0.5574	0.3884
0.463	0.5624
0.4999	0.8944
0.8021	0.4386
0.7887	0.8055
0.4738	0.2741
0.6382	0.3151
0.4675	0.3409
0.7385	0.5405

Total Caudate
Ipsilateral injected side shown
Blue= increased SBR after 24 months



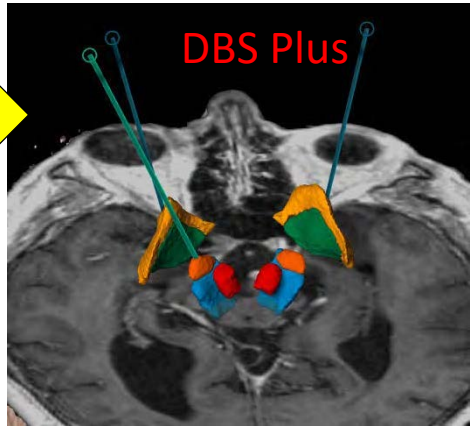
0 months	24 months
0.6758	0.3498
0.3576	0.1227
1.3565	1.3305
0.654	0.3571
1.1789	0.6422
0.21	0.387
0.7676	0.4828
0.5094	0.5173
0.7002	0.5908
0.761	0.8407
0.7604	1.3304
1.3115	0.7504
1.2919	1.1659
0.6295	0.3197
0.9597	0.5242
0.6253	0.5489
1.3014	0.836



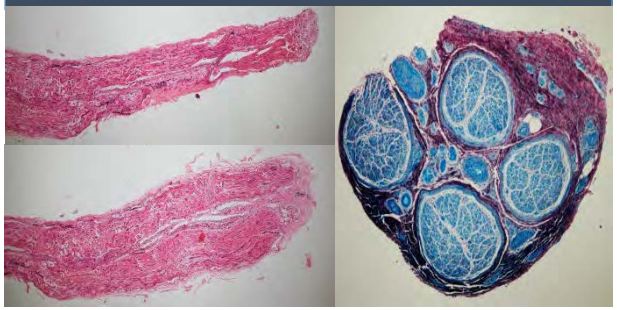
Harvest

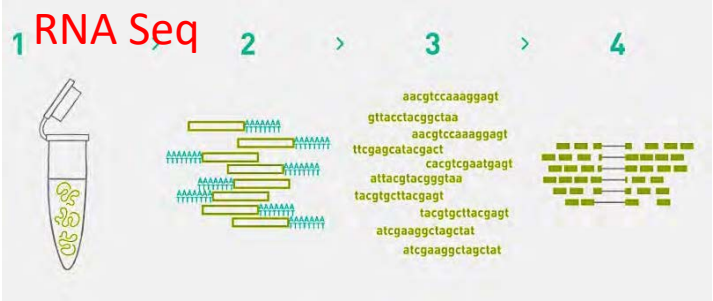
Avatar

Cell Culture



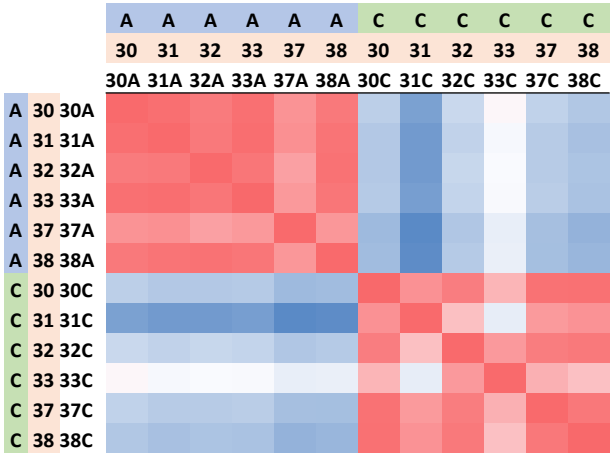
Histology





Sural Nerve RNA-seq data: Stage I vs. Stage II

- Experimental Design- 6 samples (before and after paired, 12 profiles)
  - Correlation matrix (right) suggests sharp distinction between groups, and good agreement within groups
- 15,479 total genes detected by RNA-seq
- 19 redundantly annotated genes (instance with highest largest mean expression retained)
- Decision points
  - Chose normalized over raw counts for analysis- although I am unclear on normalization procedure
    - I'm used to seeing Fragments per kilobase per million (FKPM)- this looks like counts per million (CPM), a step before FKPM, I think.
  - Calculated new values instead of the provided values for…
    - paired t-test: the provided P-value calculations were generally much smaller (possibly a Bayesian prior and/or resampling algorithm)
    - fold changes: the provided values didn't always match up to the normalized data

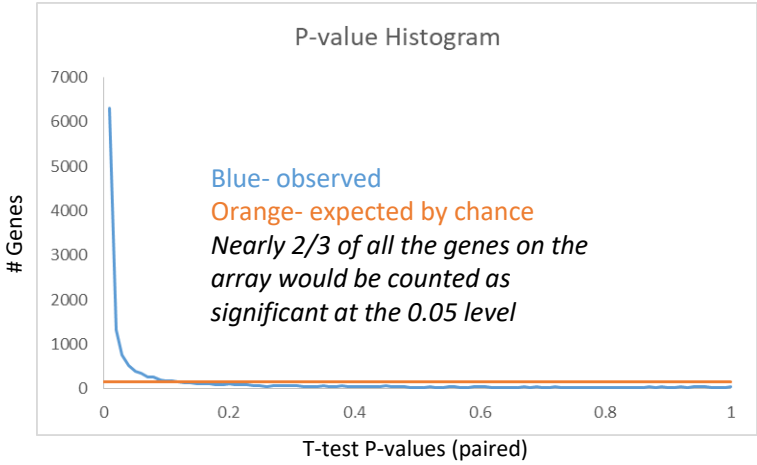


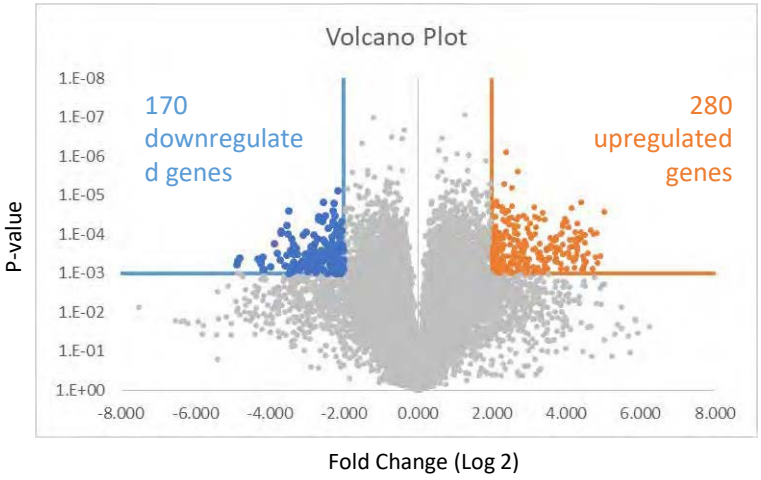
Correlation matrix (Pearson's R for every subject vs every other subject) ranges from 0.4 (blue) to 1 (red). There is strong separation by A vs C, and relatively good agreement within groups. Seeing this kind of distinction at this level usually indicates an extremely powerful effect on the transcriptional profile

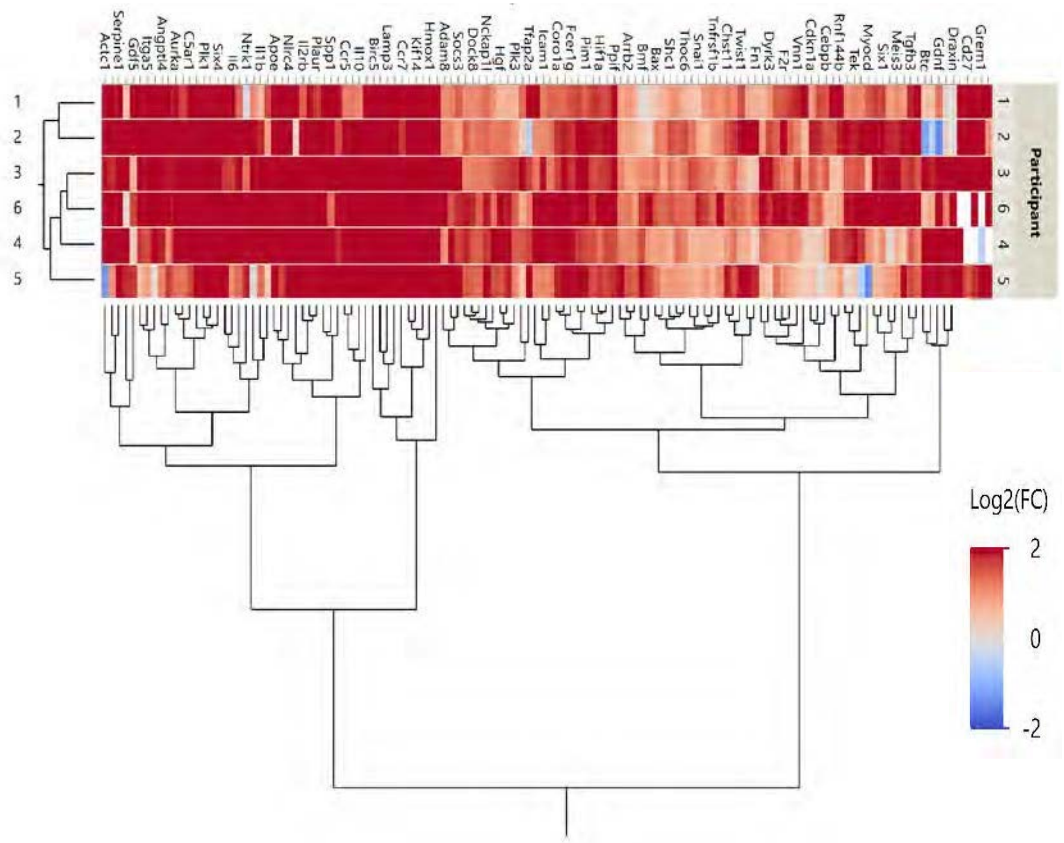
Significant Genes Affected in the Sural Nerve Grafts

- **Upper- P-value histogram for pairwise t-test results plots # genes found at different p-value cutoffs.**
  - **Chance (orange line)- the probability of finding a gene at a given p-value cutoff by the error of multiple testing**
  - **Observed (blue line)- the actual number found greatly exceeds chance at smaller p-values**
  - ***Normality, skewness are concerns (but some of the effects that violate these assumptions are so large that we aren't going to find a stats test that does NOT identify the change, although all of those tests will have some kind of a problem with the data they are testing- we may want to re-visit analysis, this is just a quick sketch***

- **Lower- 'volcano plot' of p-values (y axis, reverse log scale) vs fold change (x axis, log 2 scale)**
  - **identifies genes with large magnitude and low variance**
  - **Colored lines indicate genes significantly ($p \leq 0.0001$; $q \leq 0.0003$) upregulated (orange) and downregulated (blue) that also have a large fold change ($|FC| \leq 4$)**



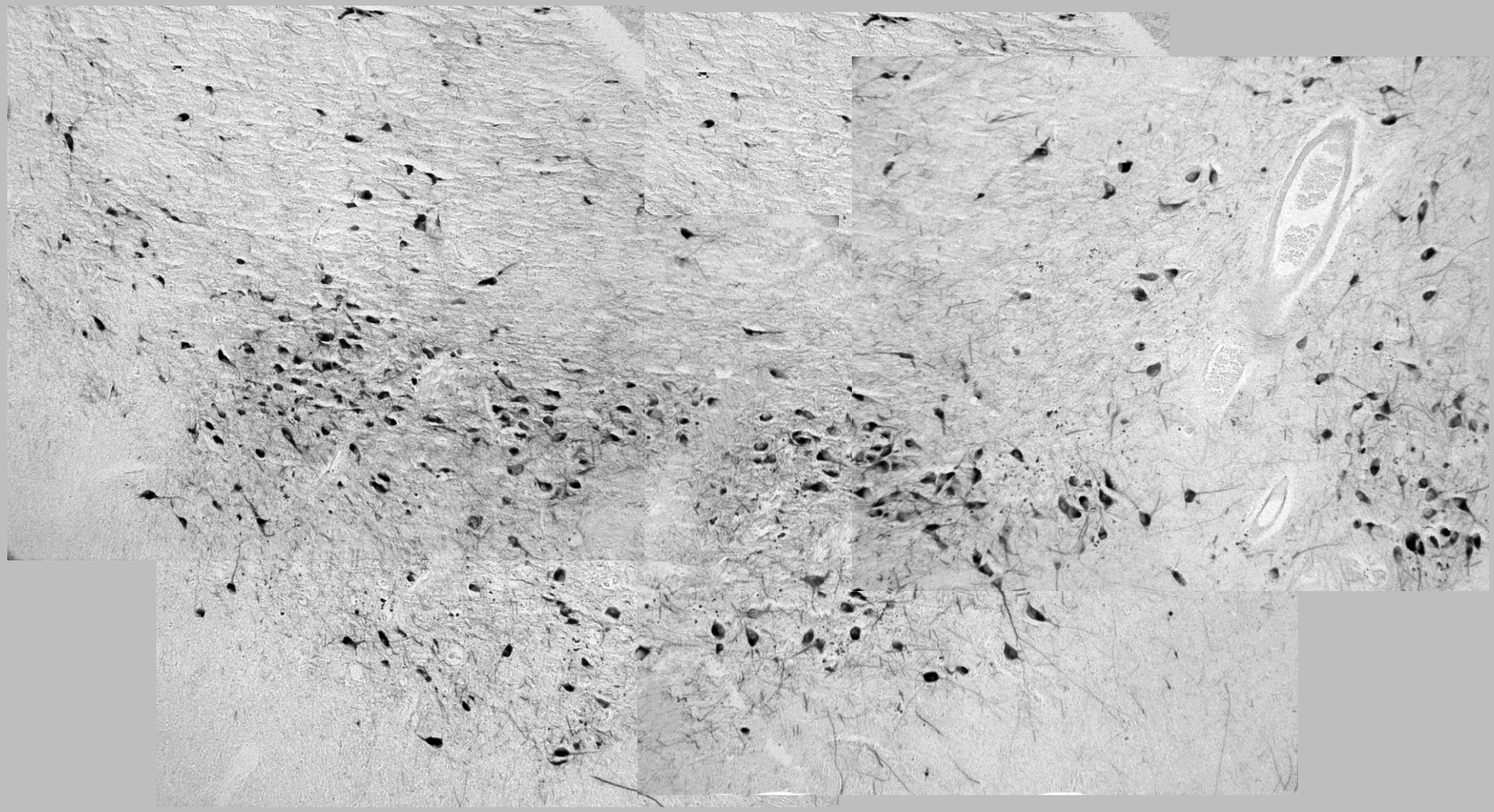




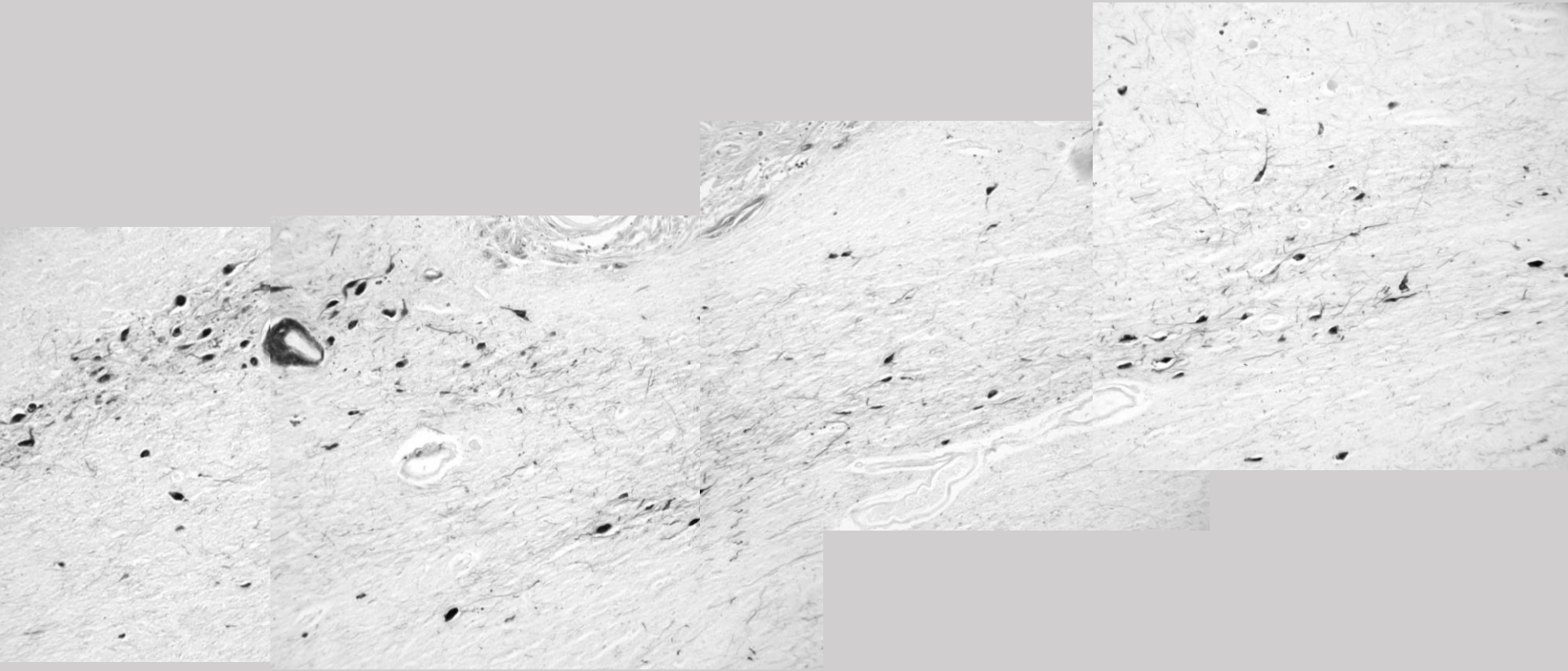
Negative Regulation of Apoptotic Processes

Postmortem Histology of SN in Subject >2 Years Post-Grafting

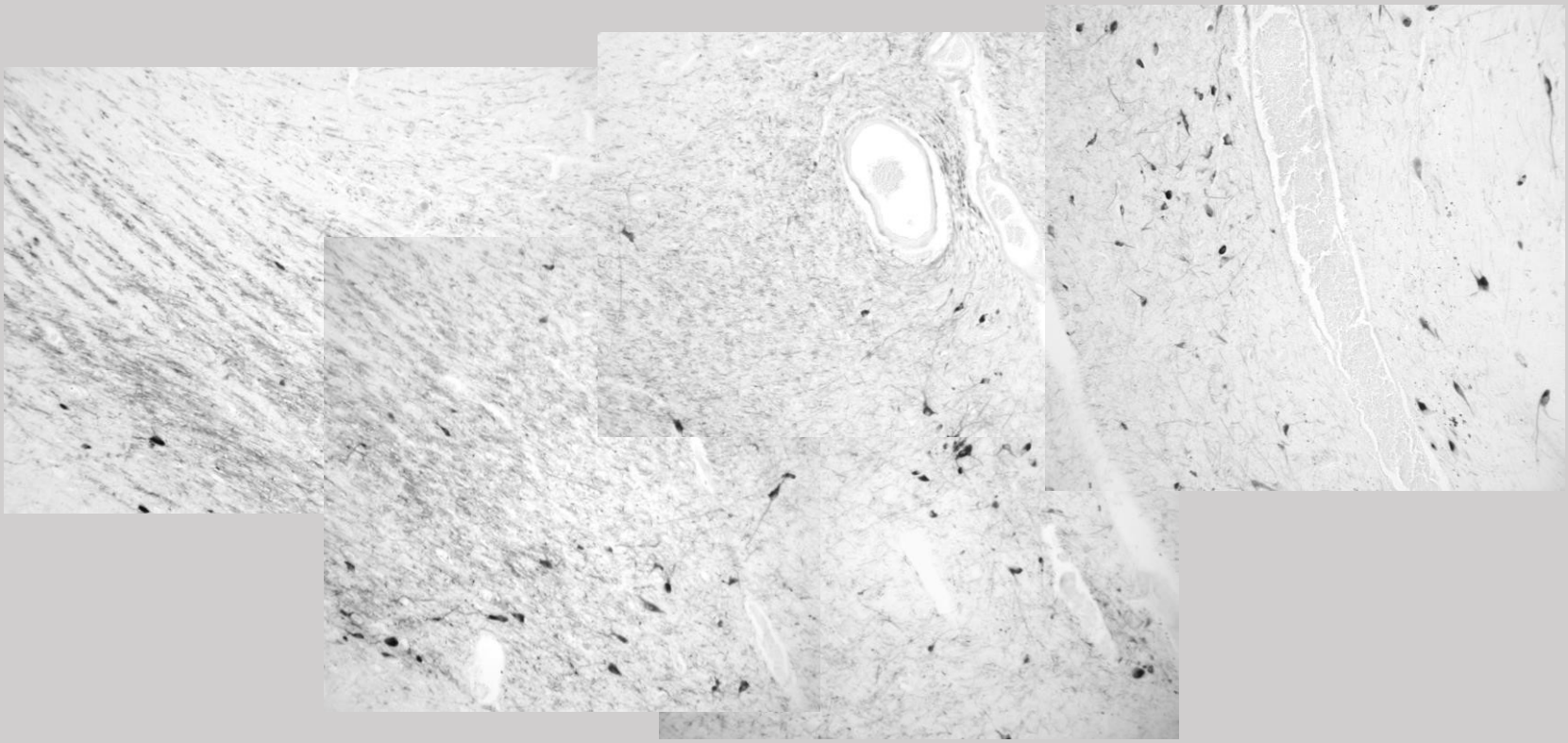
Left substantia nigra montage TH



TH Patient #1 Right side (contralateral)

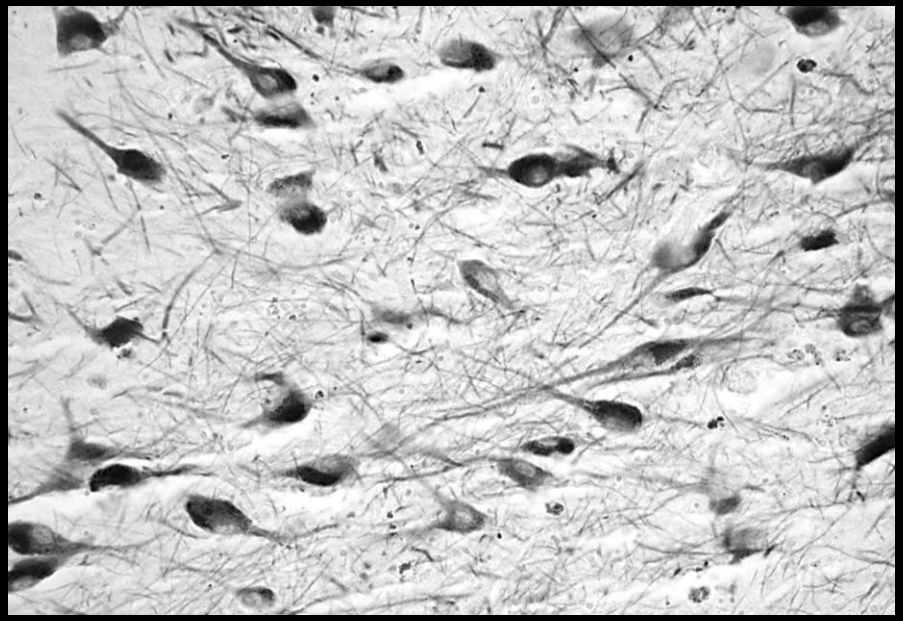


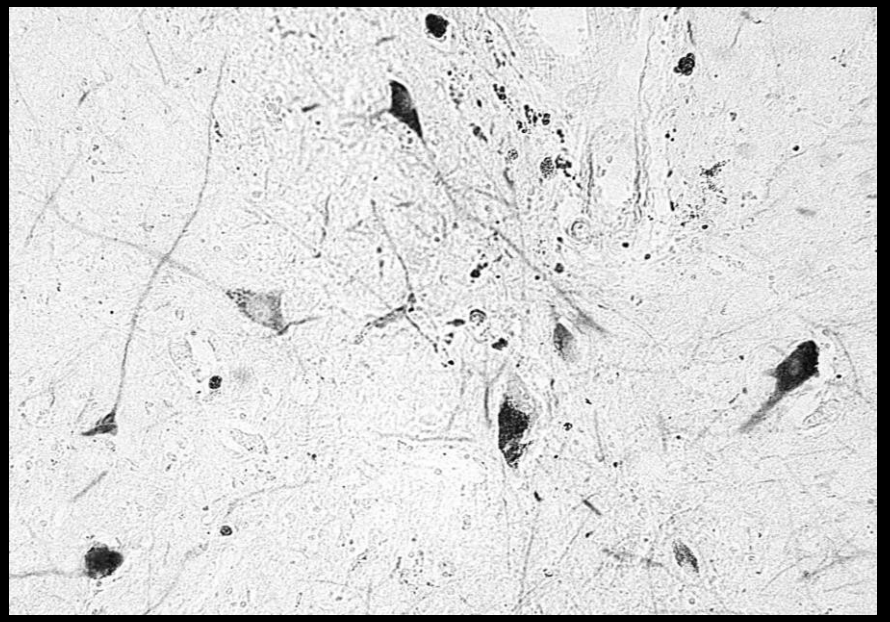
TH Patient #1 Right side (contralateral)



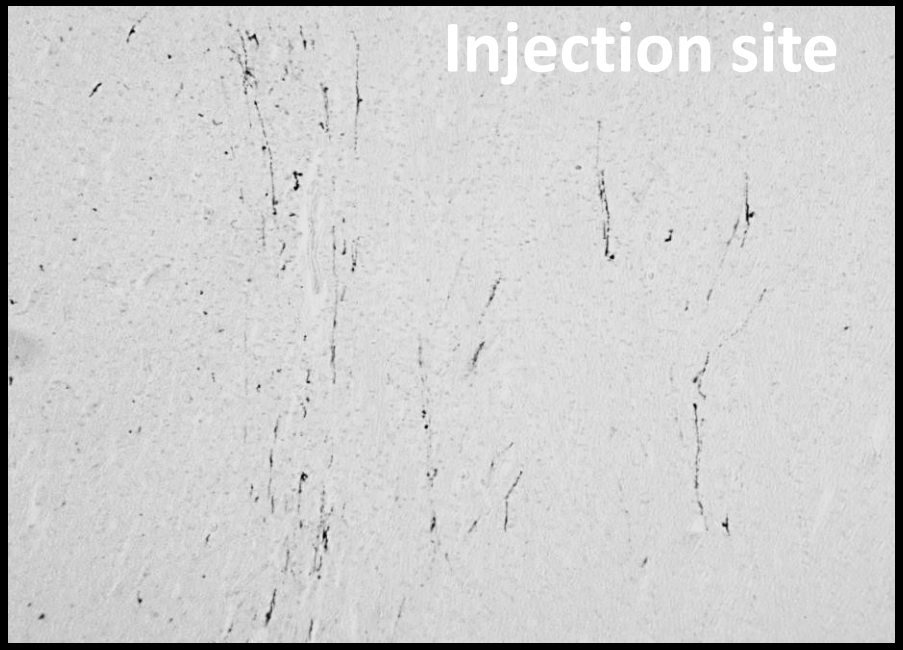
Grafted Side

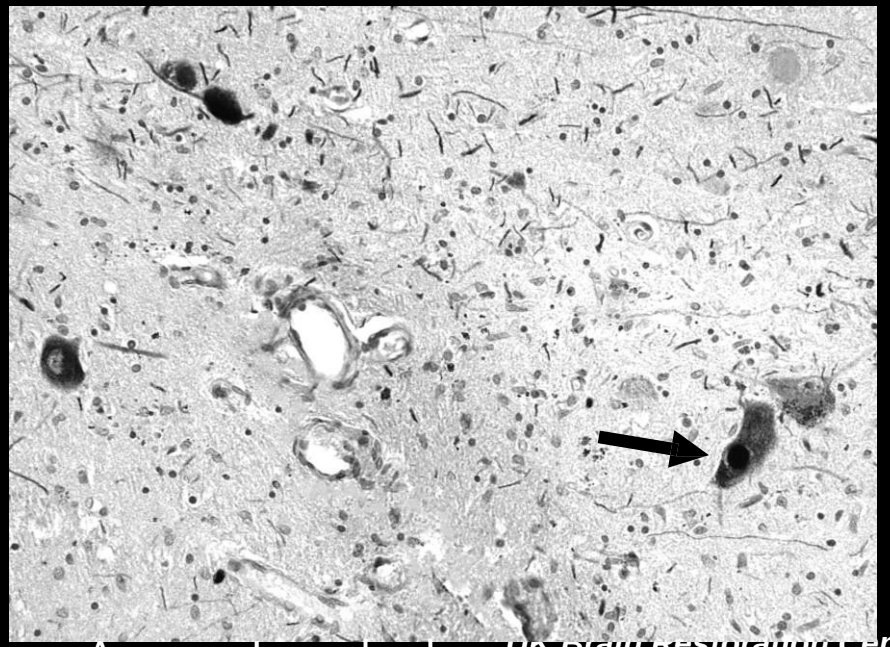
TH SN Contralateral side



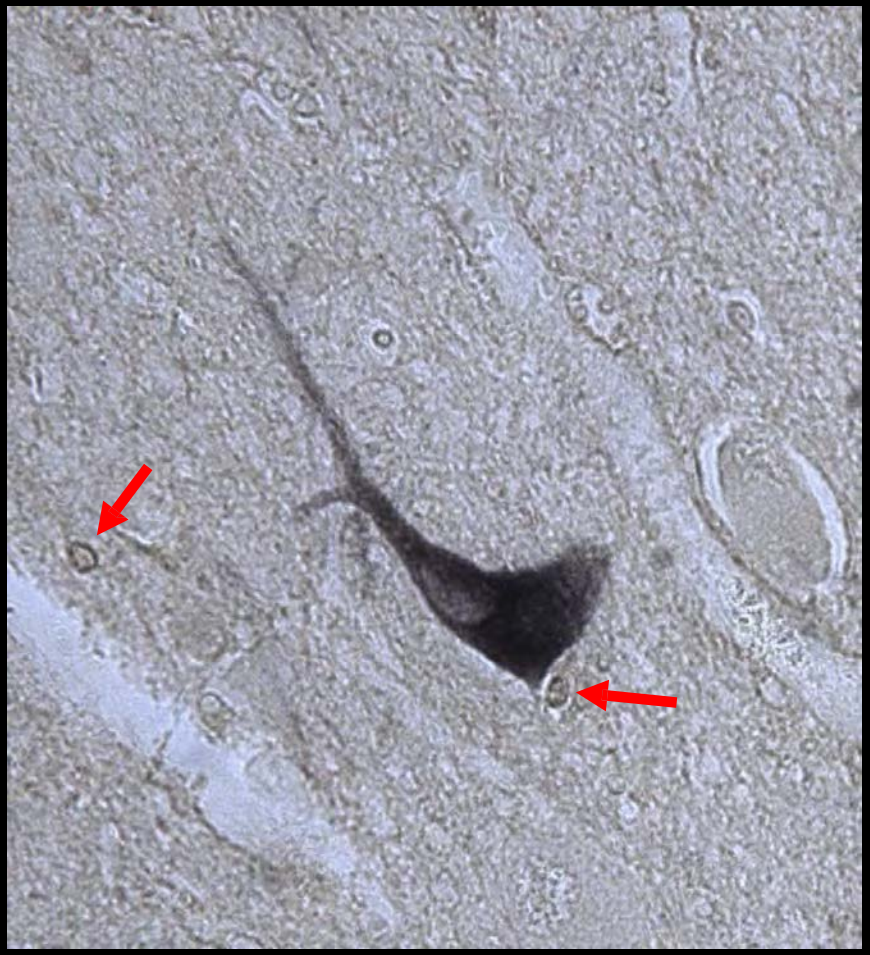


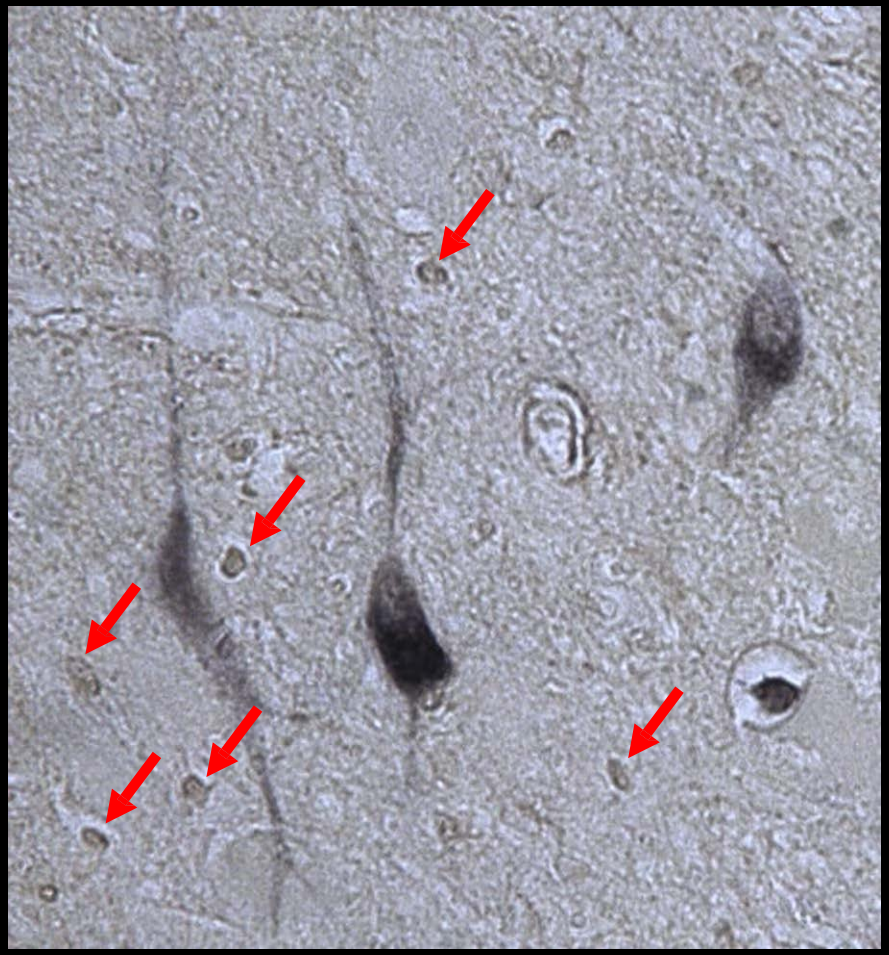
Injection site





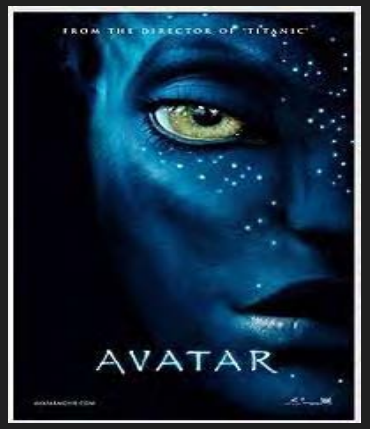
Arrow = Lewy body





S100beta and TH double labeling. Small S100beta-positive cells are seen in the vicinity of SN TH positive neurons on the transplanted side (arrows), possibly immature Schwann cells.

Neuro-Avatar:
Graft Survival

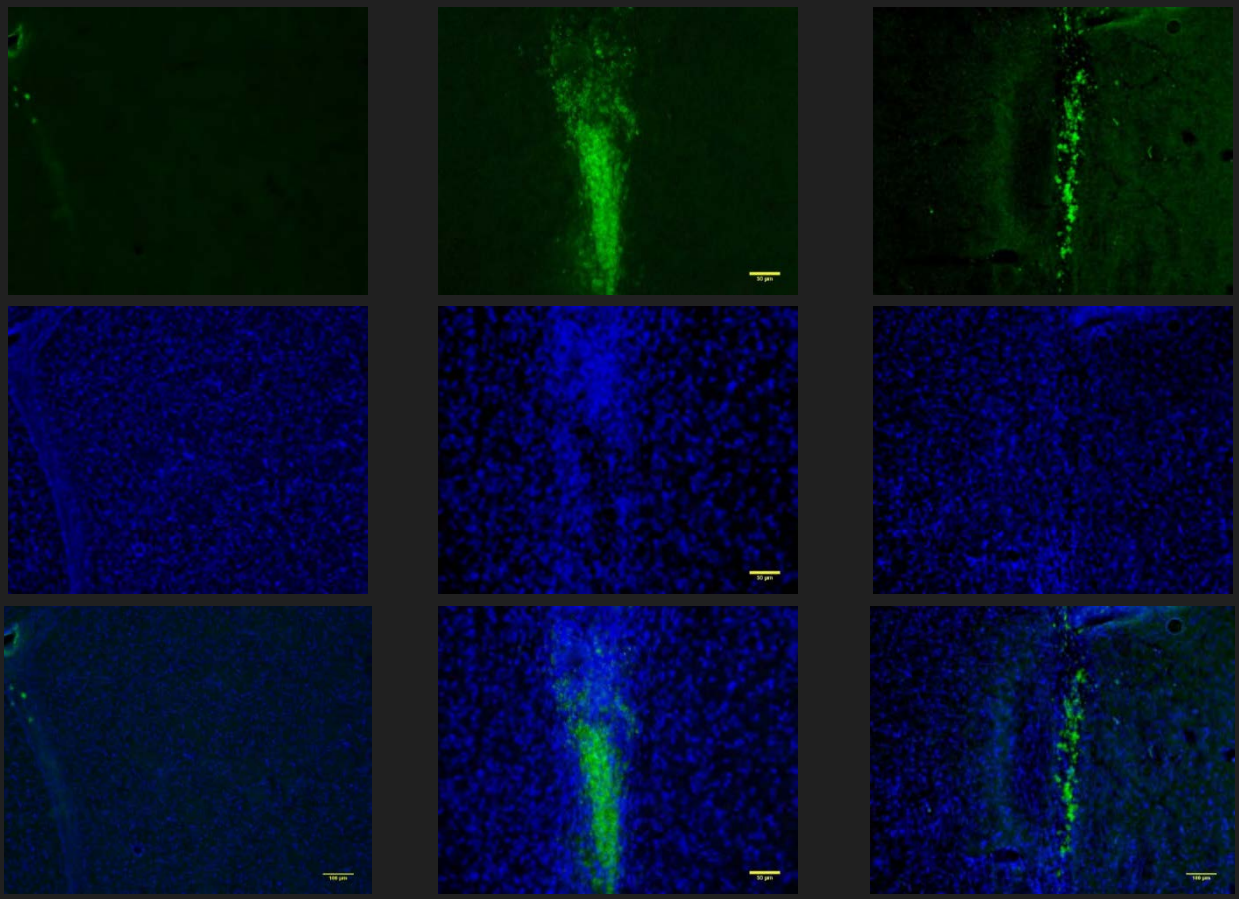


Control

Grafted

2 weeks

6 months



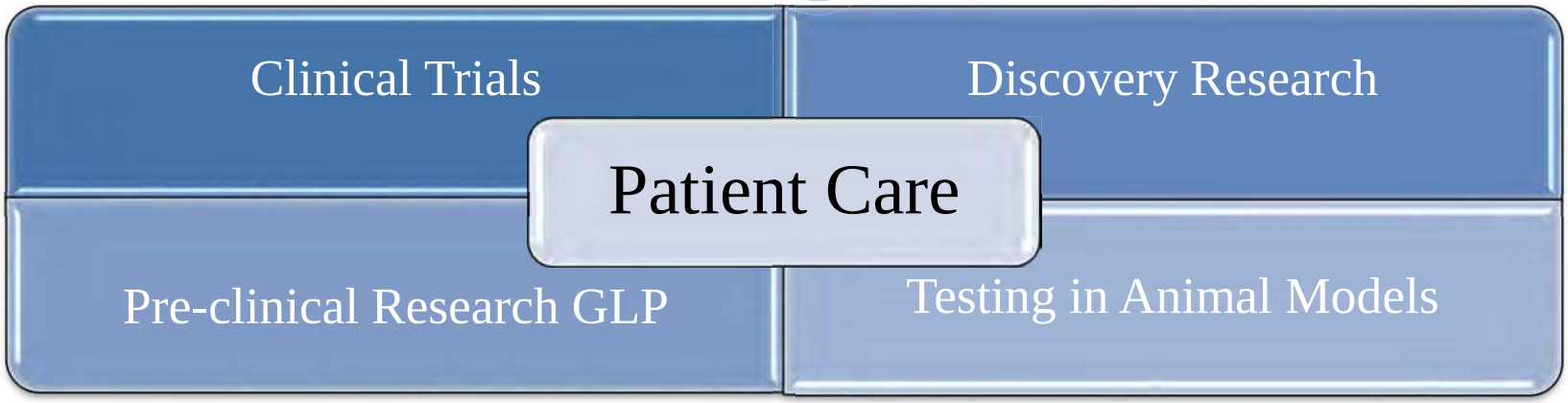
Conclusions/Future Directions:

- We currently have 74 subjects with grafts into SN (66) or NBM (8). No Safety issues to date.
- Phase I trials continuing to determine safety and best implantation procedures and methods. Now have preliminary studies with up to 20 pieces in 4 tracks (dose ranging and safety).
- Phase IIa blinded trial under development.
- Parallel studies in nude rats (“sural nerve Avatars”) in vivo and in oculo, cell culture, RNAseq, and proteomics of the Phase I and II sural nerve samples are underway and show viability of human tissues in nude rats and new clues to the repair process.
- Three subjects have passed away from natural causes at 2,2.5 and 2.75 years after transplantation – studies are ongoing to determine the effects of the sural nerve grafts and determine how they function.

Safety

- No severe adverse events attributable to graft or graft procedure.
- Adverse event profile similar to DBS surgery without grafting.

UK Brain Restoration Center: Strategic Focus



- Clinical Trials focus on advancing patient care
- Pre-clinical research provides initial safety data and establishes protocol design (GLP level capabilities)
- Animal research validates clinical findings
- Discovery research identifies potential novel therapies


Ann Hanley
Parkinson's Research Fund

First Affiliated Hospital of Zhengzhou University July 2016: First DBS Implant: Sponsored by Medtronic and PINS

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Special Thanks

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ACKNOWLEDGEMENTS

Participating Scientists

- Dr. Luke Bradley
- Dr. Richard Grondin
- Dr. Zhiming Zhang
- Dr. Yi Ai
- Peter Huettl
- Francois Pomerleau
- Dr. Ofelia Littrell

Funding

- NINDS NS39787

(Udall Center, 1999-2012)

- NIH CTSA Award
- Medtronic, Inc.
- Braden-Clark Endowment
- Dupree Parkinson's Fund
- Ann Hanley Fund


Black Tip Sharks in Palau Micronesia 2013