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75th Meeting of the Cellular, Tissue, & Gene Therapies Advisory Committee (CTGTAC)
Virtual September 27, 2023

Some guiding principles when contemplating stem cell-based approaches to neurological disease

Evan Y. Snyder, MD, PhD, FAAP

Professor; Founding Director, Center for Stem Cells & Regenerative Medicine,
Founding Director, Stem Cell Research Center & Core Facility
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Sanford Burnham Prebys Medical Discovery Institute (SBP)
Faculty Physician, School of Medicine, University of California, San Diego (UCSD)
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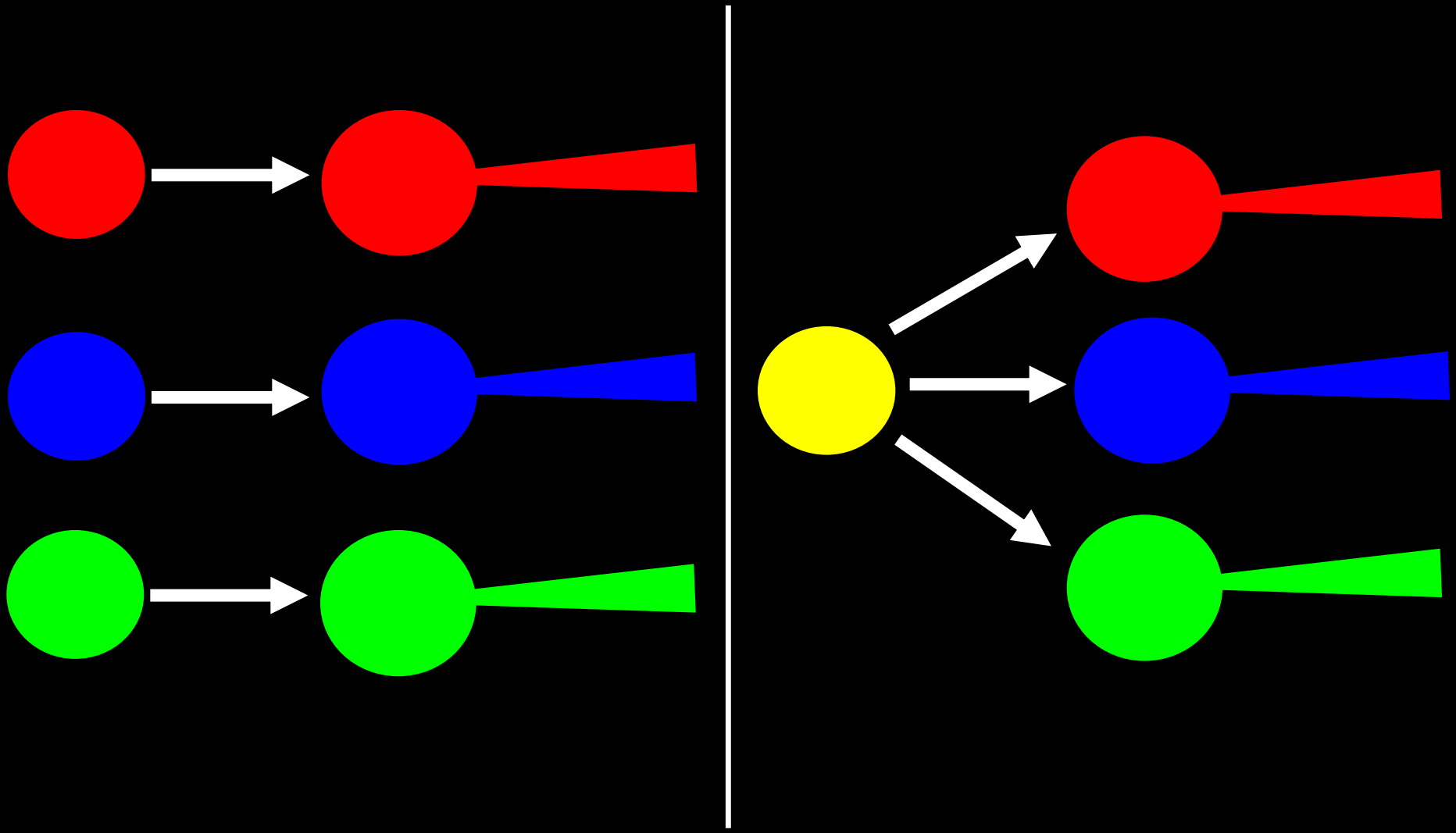
Founding Coordinator, Southern California Stem Cell Consortium
Founding Member, Steering Committee, Sanford (San Diego) Consortium for Regenerative Medicine (SCRM)
Member, Sanford Child Health Research Center
Chairman, Scientific Advisory Committee, National Institute of General Medical Sciences Human Genetic Cell Repository for NIH
Former Chairman, FDA's Cell, Tissue, & Gene Therapy Advisory Committee

COI Disclosure: Consultant for *Domina Therapeutics*; *Funding*: NIH; CIRM; International Rett Found.; CPARF

When is it appropriate to attempt a therapy in the face of imperfect and/or incomplete knowledge?

- Standard-of-care is suboptimal or no therapy/cure exists
- If the biological data make sense when subjected to critical scrutiny
  - Preclinical findings are consistent with our knowledge of
    - the cell's biology
    - the disease's pathophysiology or processes known to drive it
- If "better-proven" options are not jeopardized
    - e.g., irradiation for brain tumors
- If it is safe
- If one does no harm

The power of the stem cell field was that it changed our thinking from the rigid deterministic model of biology & disease, as depicted on the left, to a more "plastic"/"flexible" view depicted on the right *(within limits)*



Stem cells are components of

Intrinsic developmental programs for

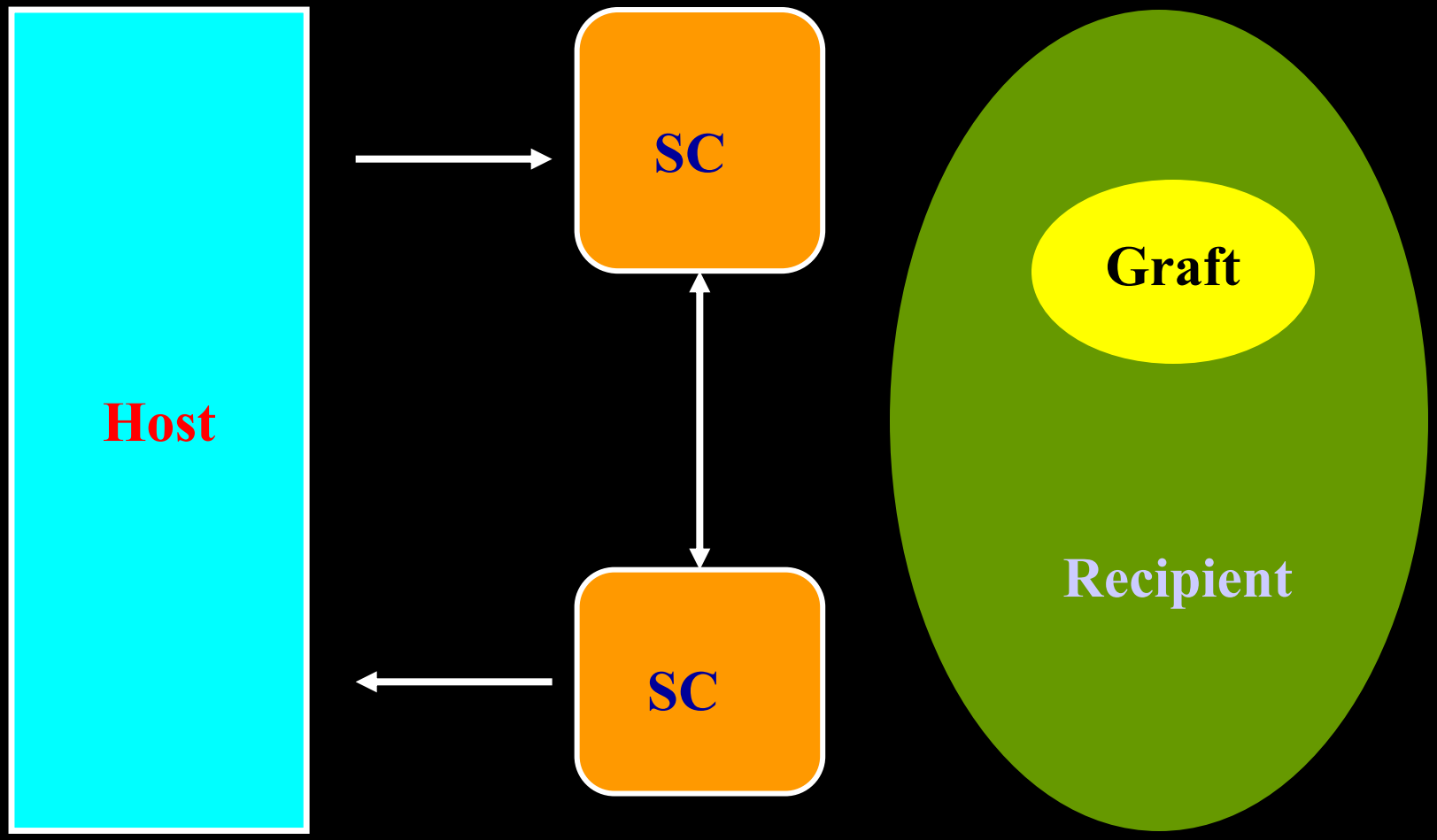
- Putting system together *("organogenesis")*
- Maintaining its balance throughout life, even in the face of perturbations *("homeostasis")*

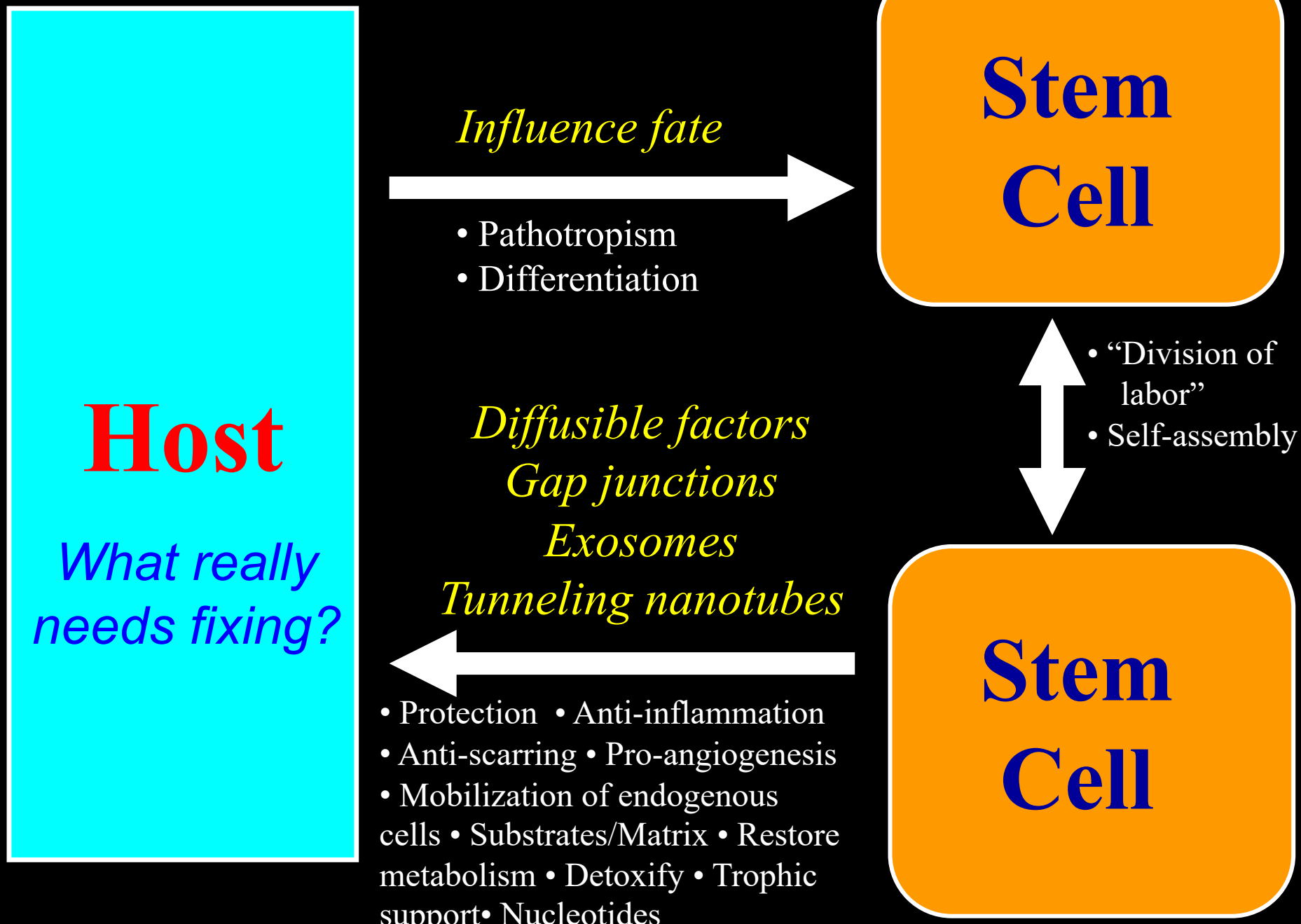
Stem Cells Model Development

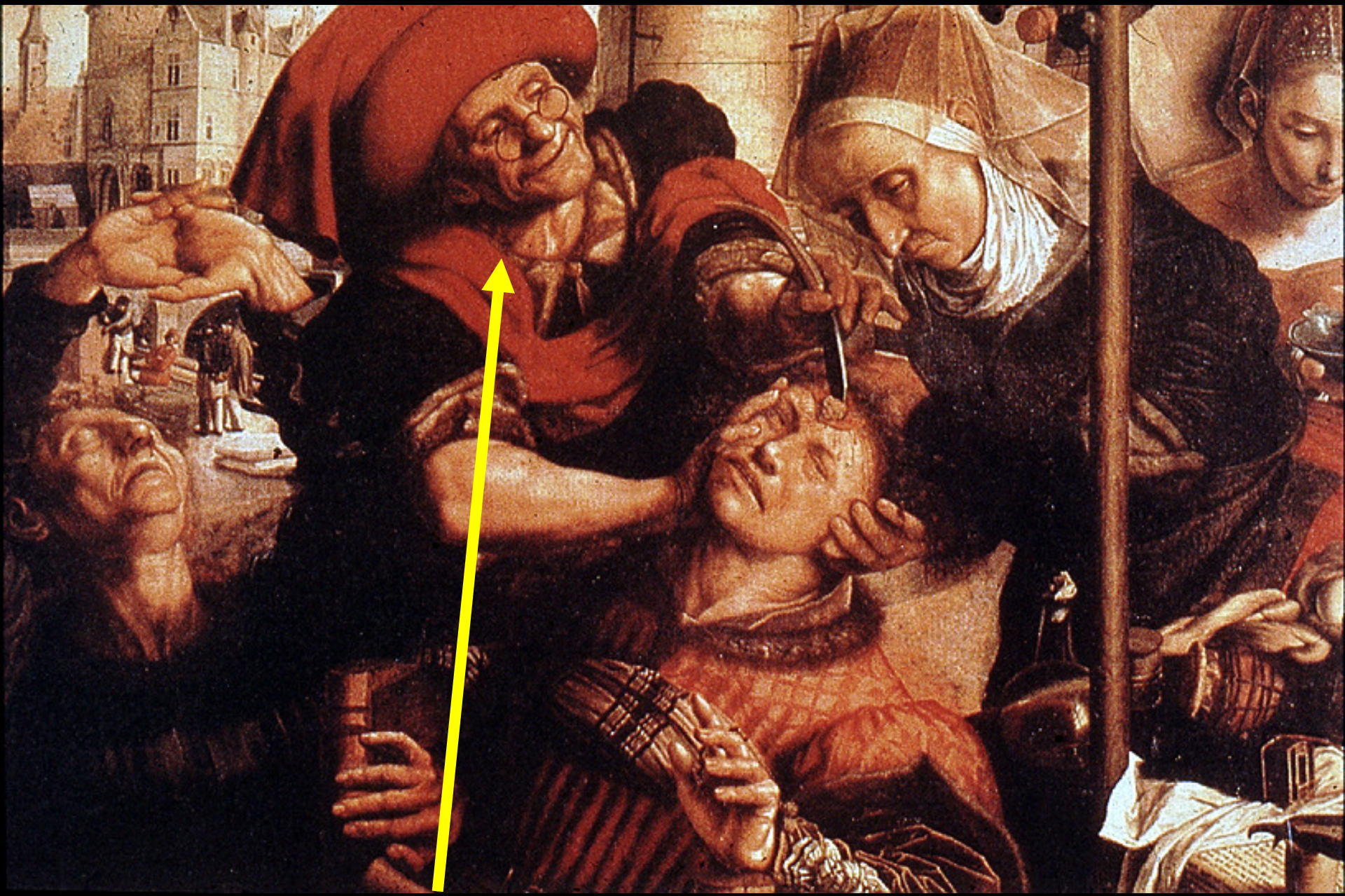
These are the programs we hope to invoke or re-invoke or harness or exploit

– learn, understand, & respect these

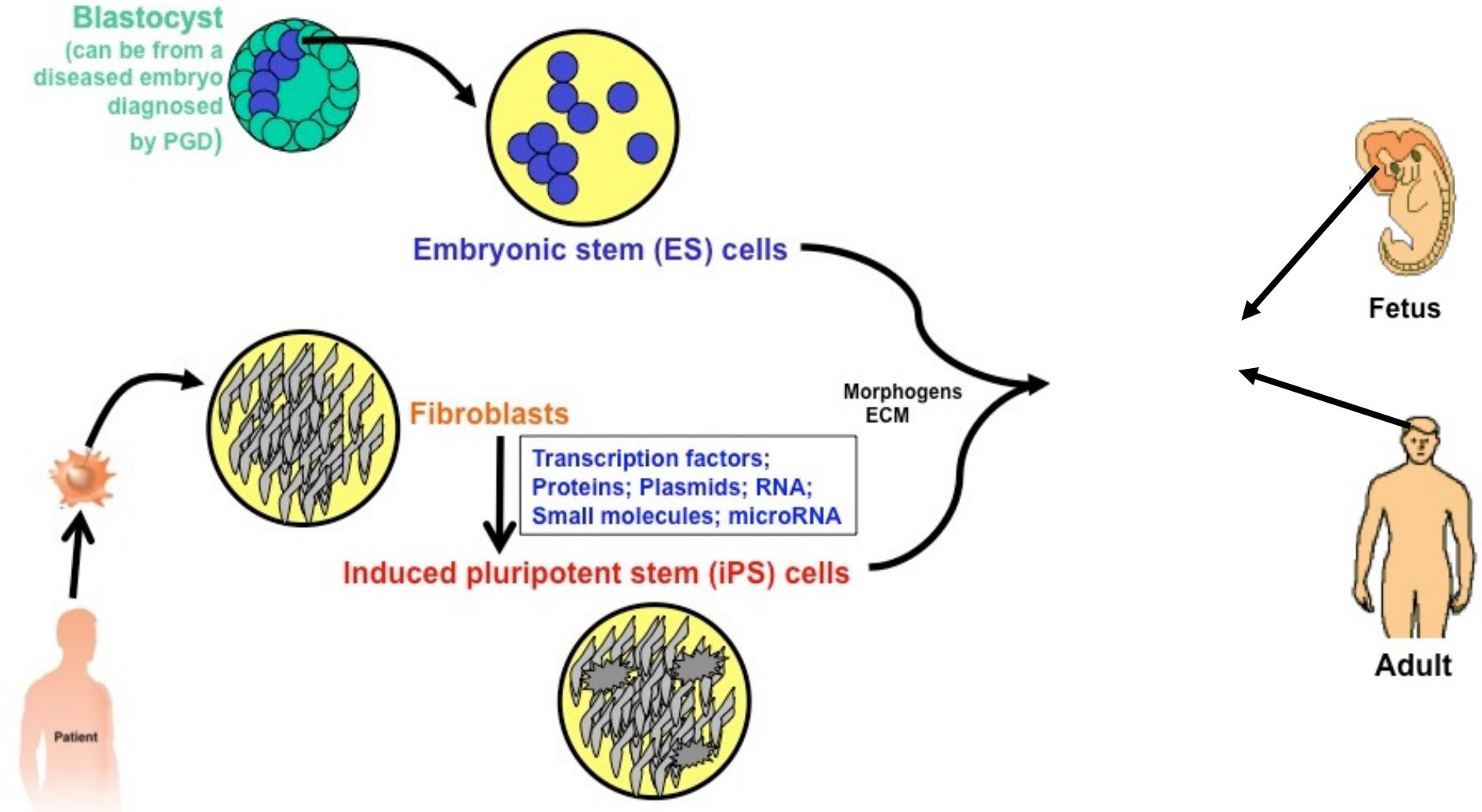
Dialogue between stem cell & recipient




Host
What really needs fixing?
Influence fate
• Pathotropism
• Differentiation
Stem Cell
• "Division of labor"
• Self-assembly
Diffusible factors
Gap junctions
Exosomes
Tunneling nanotubes
• Protection • Anti-inflammation
• Anti-scarring • Pro-angiogenesis
• Mobilization of endogenous cells • Substrates/Matrix • Restore metabolism • Detoxify • Trophic support• Nucleotides
Stem Cell

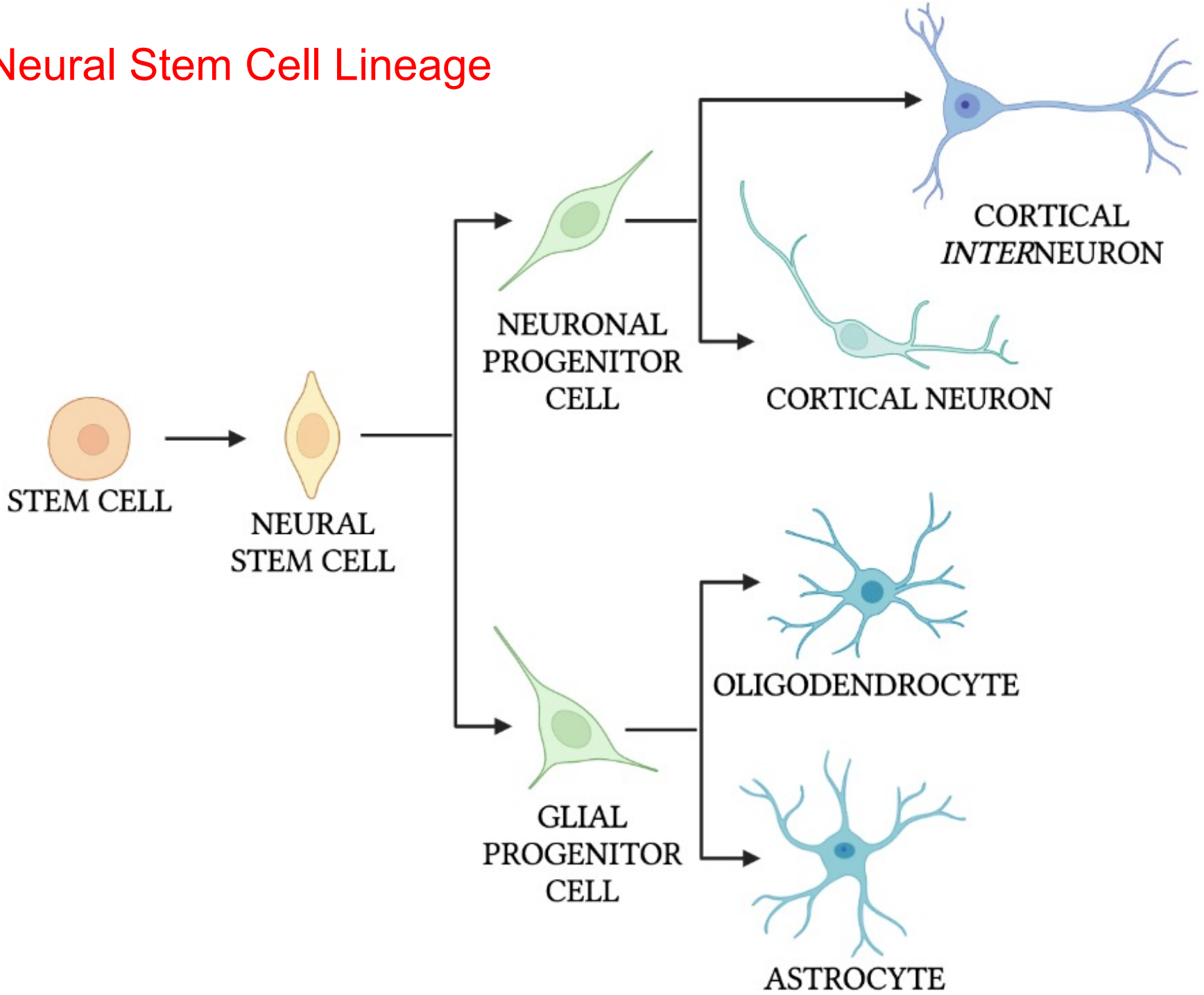


"Translational Developmental Biology"


Blastocyst
(can be from a diseased embryo diagnosed by PGD)
Embryonic stem (ES) cells
Fetus
Fibroblasts
Morphogens
ECM
Transcription factors;
Proteins; Plasmids; RNA;
Small molecules; microRNA
Induced pluripotent stem (iPS) cells
Adult
Patient

cord cells)

Neural Stem Cell Lineage

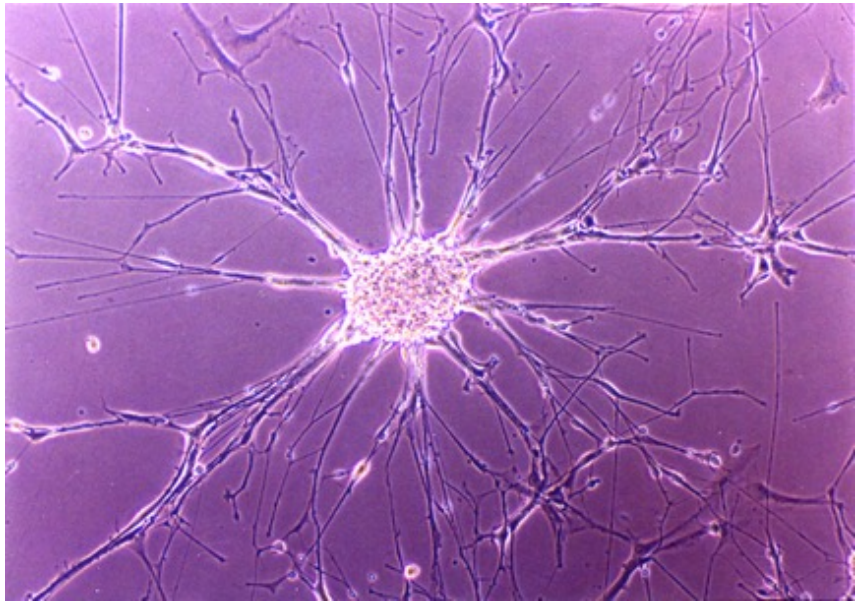


- Know the biology of the cell
  - Does it adhere to normal developmental rules & respond to cues?
    - Including in the adult?

nature biotechnology

Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes

Jonathan D. Flax[1], Sanjay Aurora[1], Chunhua Yang, Clemence Simonin, Ann Marie Wills, Lori L. Billinghurst, Moncef Jendoubi[1], Richard L. Sidman[2], John H. Wolfe[3], Seung U. Kim[4], and Evan Y. Snyder*

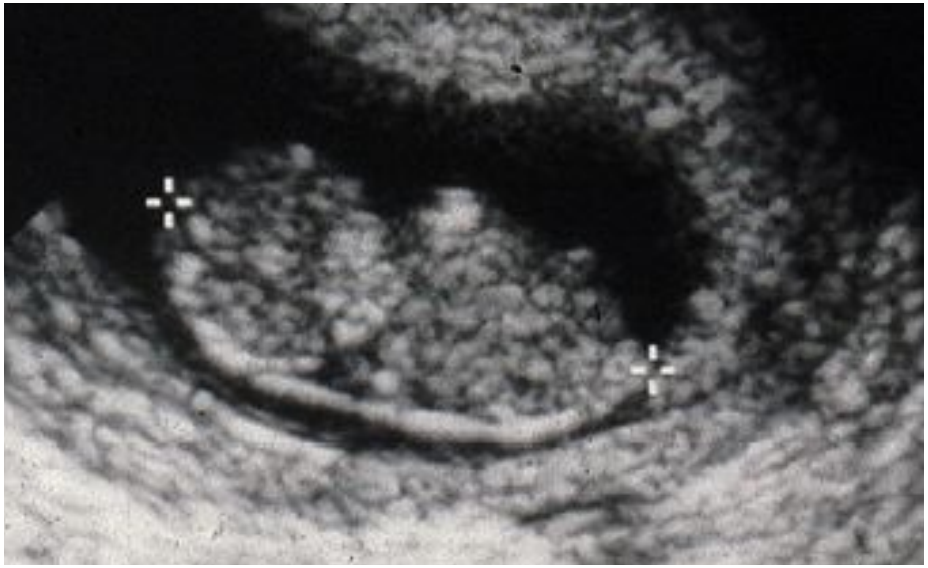


Flax et al, *Nature Biotech*, 1998

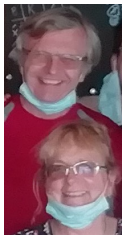
Science
AAAS
7 SEPTEMBER 2001 VOL 293 SCIENCE www.sciencemag.org

Segregation of Human Neural Stem Cells in the Developing Primate Forebrain

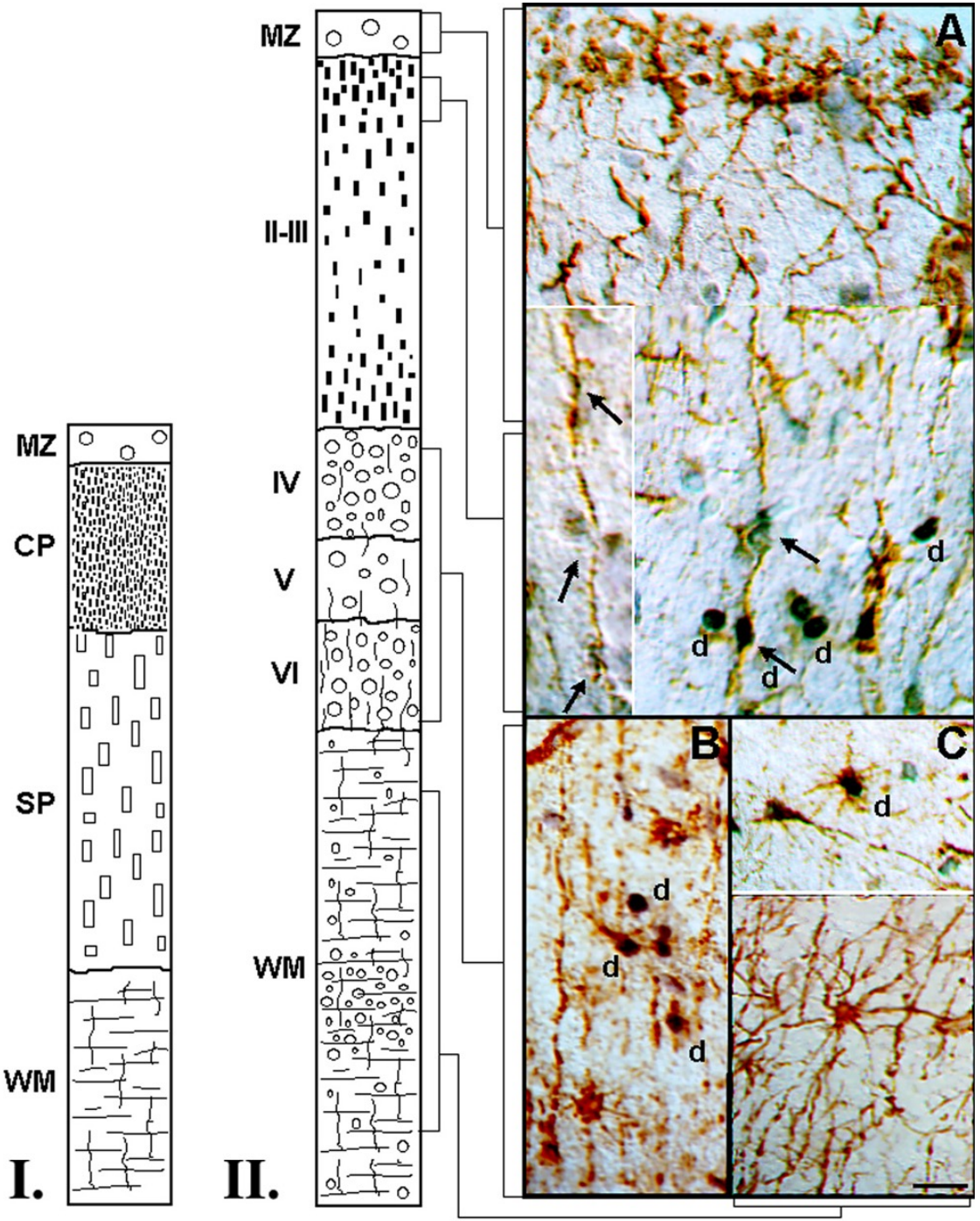
Václav Ourednik,[1*†] Jitka Ourednik,[1*] Jonathan D. Flax,[1]
W. Michael Zawada,[2] Cynthia Hutt,[2] Chunhua Yang,[1]
Kook I. Park,[1,3] Seung U. Kim,[4] Richard L. Sidman,[5]
Curt R. Freed,[2‡] Evan Y. Snyder[1†‡]



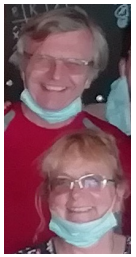
Ultrasound of fetal monkey; hNSCs injected into ventricles, quickly & safely ➔integration into developing cortex



Vaclav & Jitka Ourednik

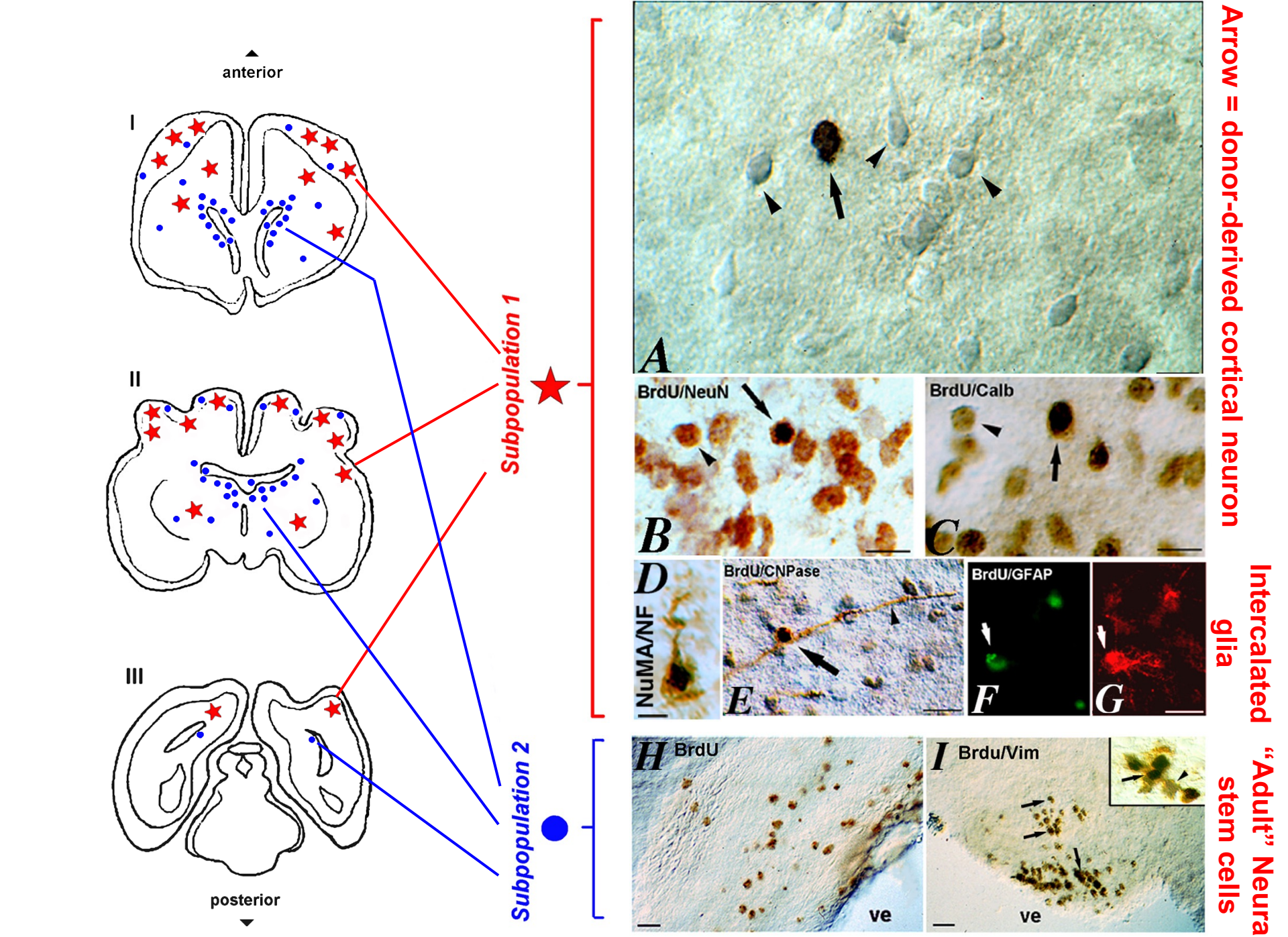


donor hNSC derivatives = *black nuclei*



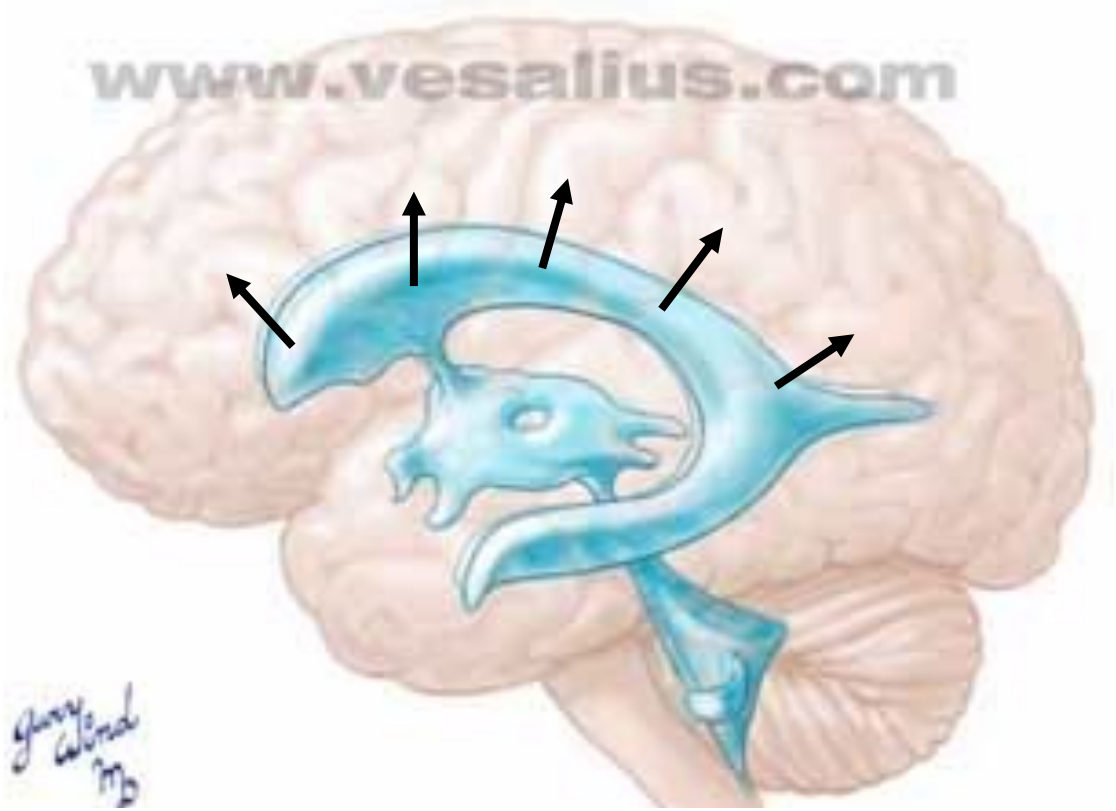
Vaclav & Jitka Ourednik

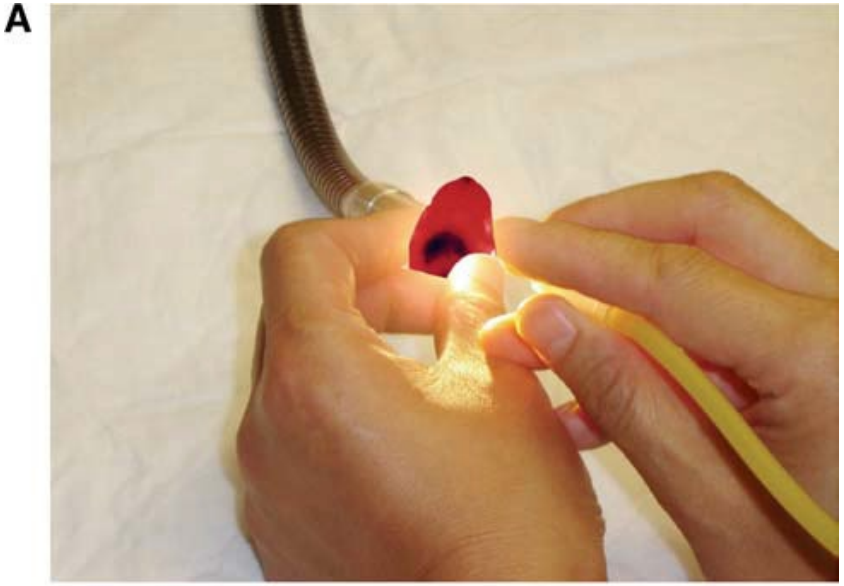
Ourednik et al, *Science*, 2001

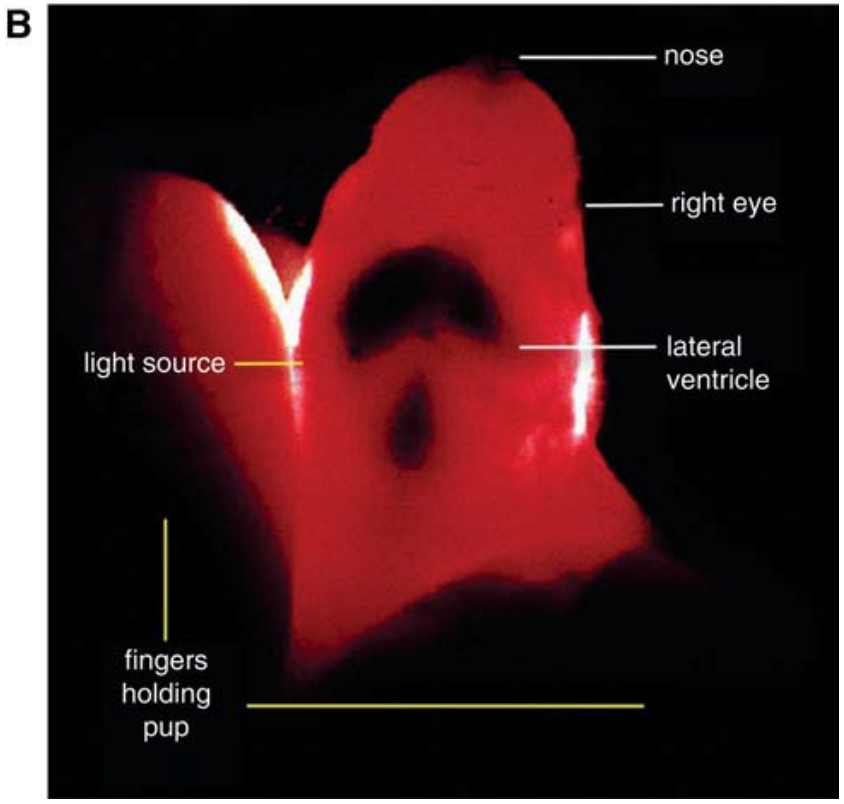


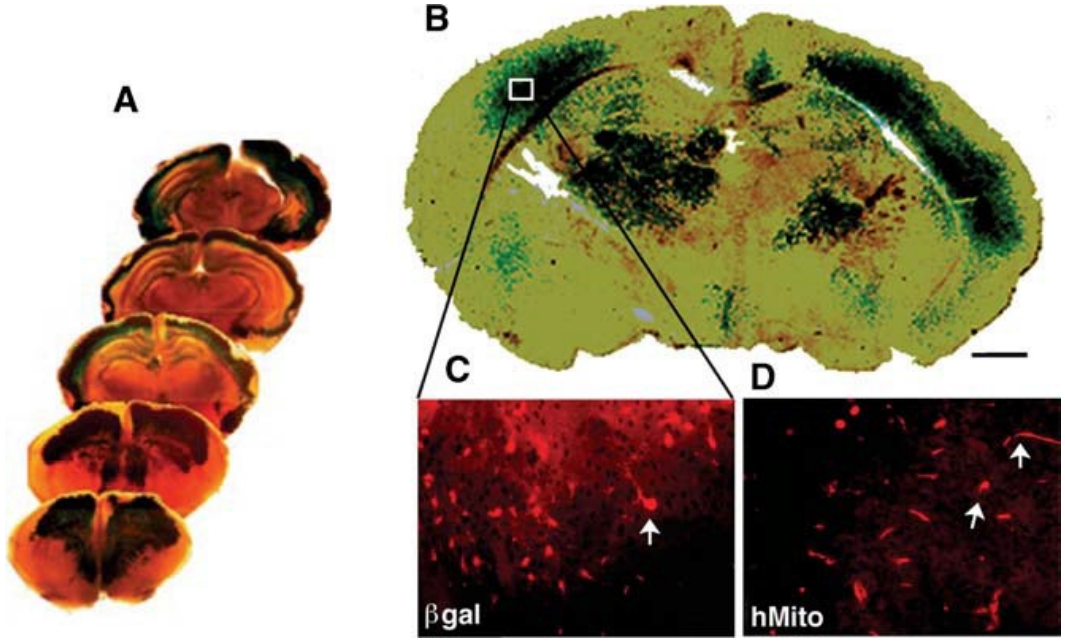
Ourednik et al, *Science*, 2001

- Does the normal developmental program of the cell fill a known therapeutic gap or suggest a therapeutic strategy?

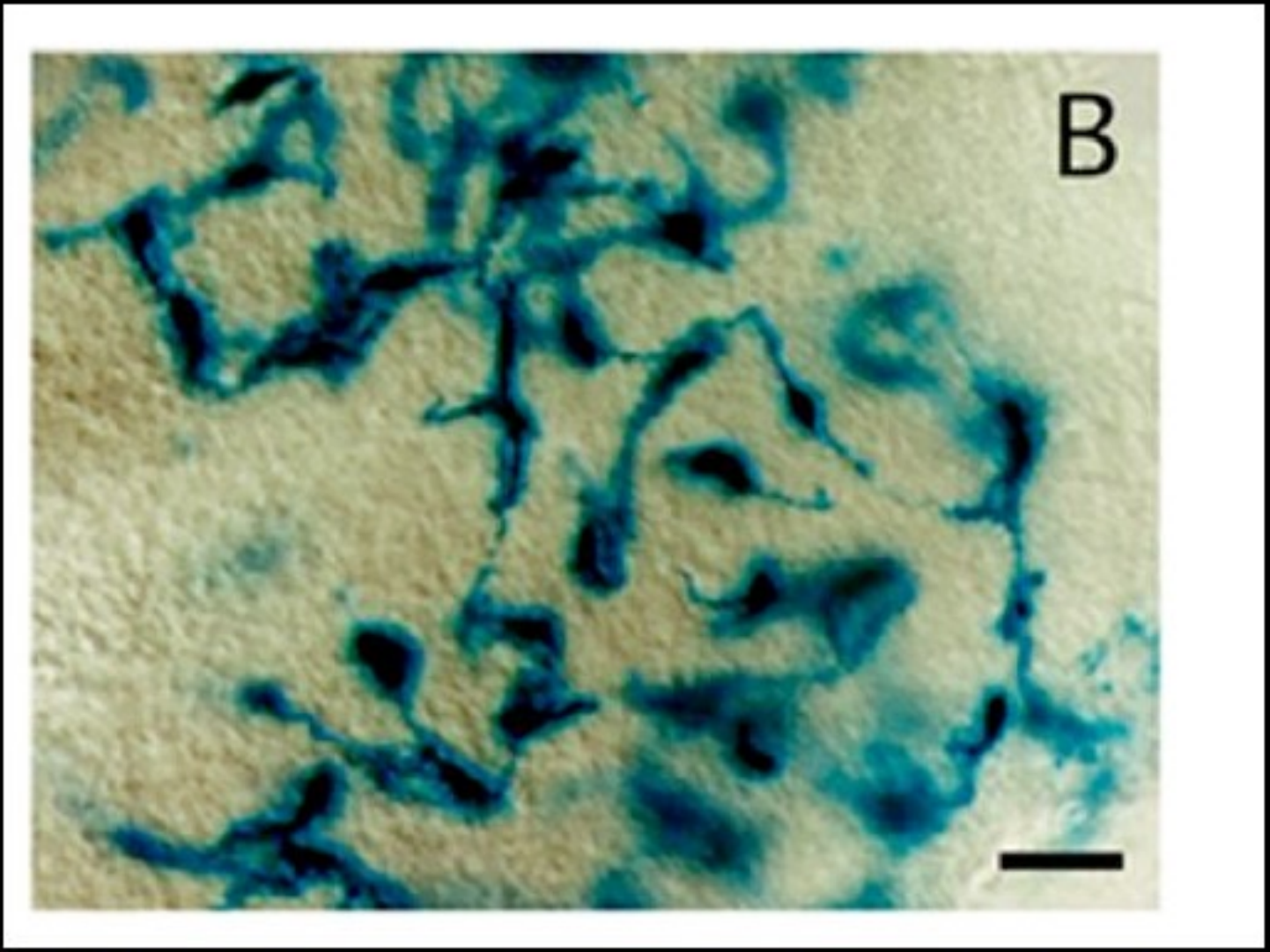

www.vesalius.com







Lee J-P et al, *Nature Med* (2007), *Curr Protoc Neurosci* (2008)



Multiple cells types spontaneously emerge, integrate, "talk to each other", "talk" to "white" host cells, & express a foreign gene (*lacZ*-blue)

Lysosomal Storage Disorders

Tay-Sachs/ Sandhoff disease

Providing normal cross-corrective lysosomal enzyme to most of the brain was challenging at the time

nature

Neural progenitor cell engraftment corrects lysosomal storage throughout the MRS VII mouse brain

Evan Y. Snyder*, Rosanne M. Taylor†‡ & John H. Wolfe†§

1. *Departments of Neurology and Pediatrics, Harvard Medical School, Children's Hospital, Boston, Massachusetts 02115, USA
2. †Laboratory of Pathology and Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
3. ‡Present address: Department of Animal Science, University of Sydney, Sydney, Australia.

Nature 374, 367-370 (23 March 1995)

Could Neural Stem Cells Rescue Mice with a Neurodegenerative Disease by Globally (brain-wide) Replacing the Enzyme it Lacks

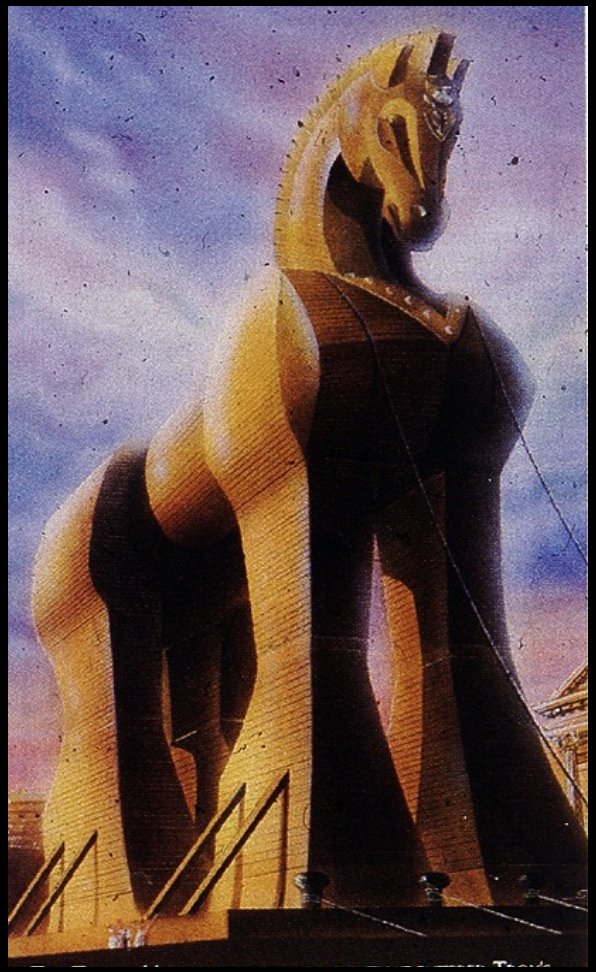
YES

(MPS VII Mouse)

(β-glucuronidase)

Rosanne Taylor

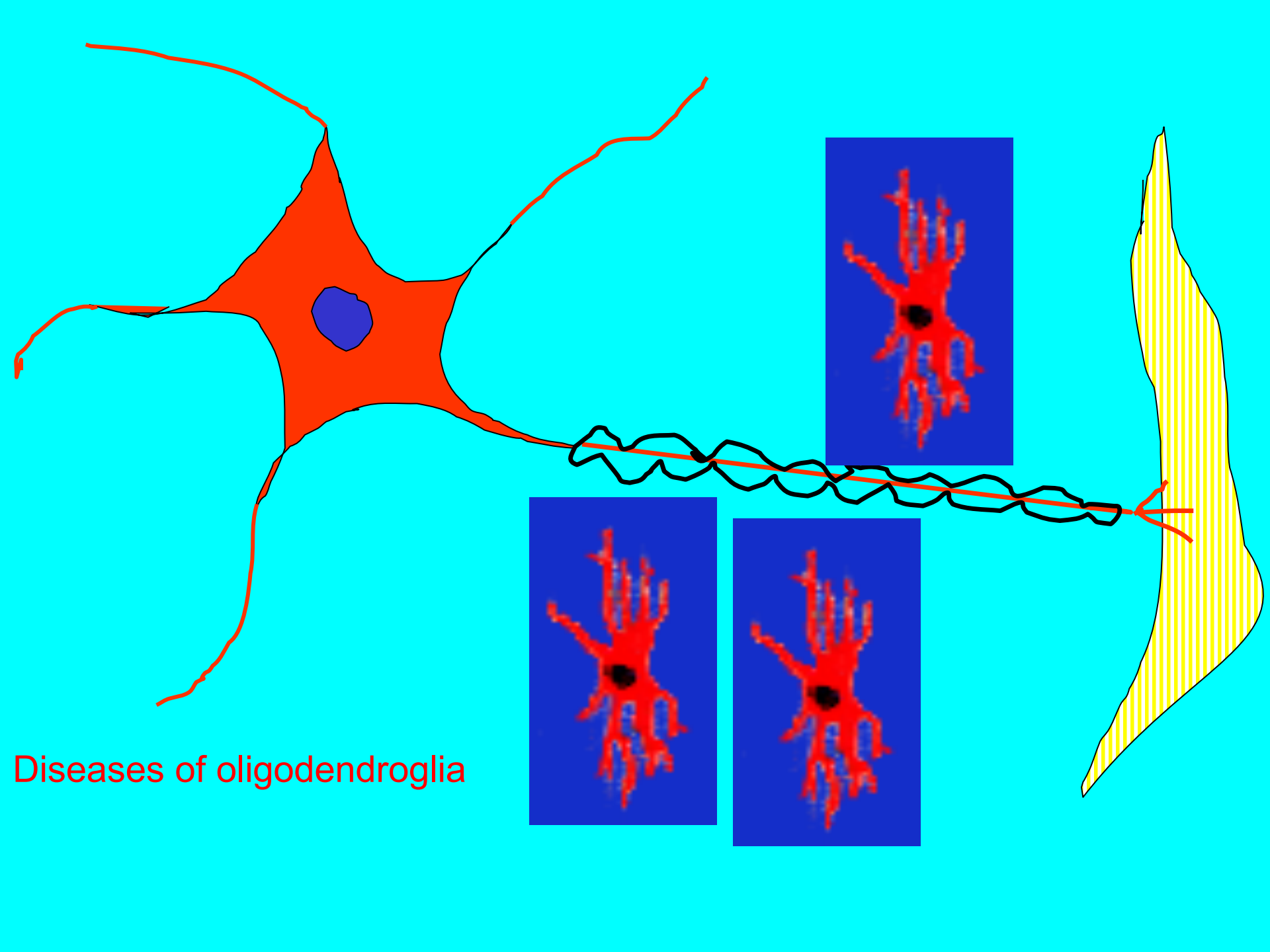
John Wolfe



Encouraging global *gene* product replacement by “piggy-backing” on a normal developmental process with “normal” developmental cells

What about global *cell* replacement?

Yes –
for a neural cell type predominantly born *postnatally* during normal CNS development

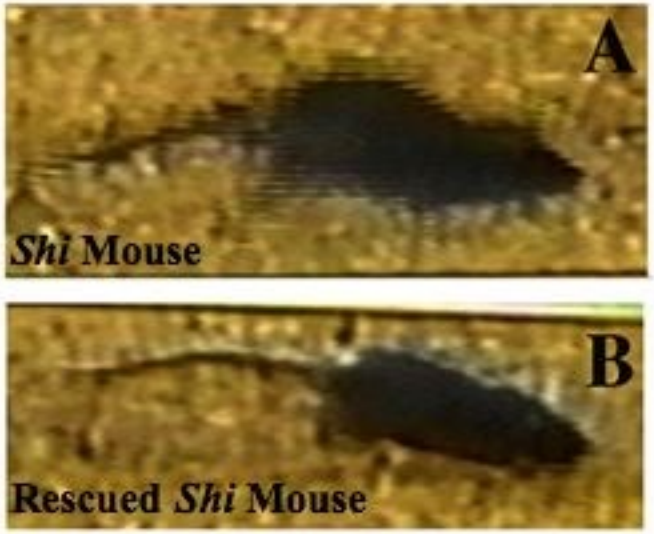


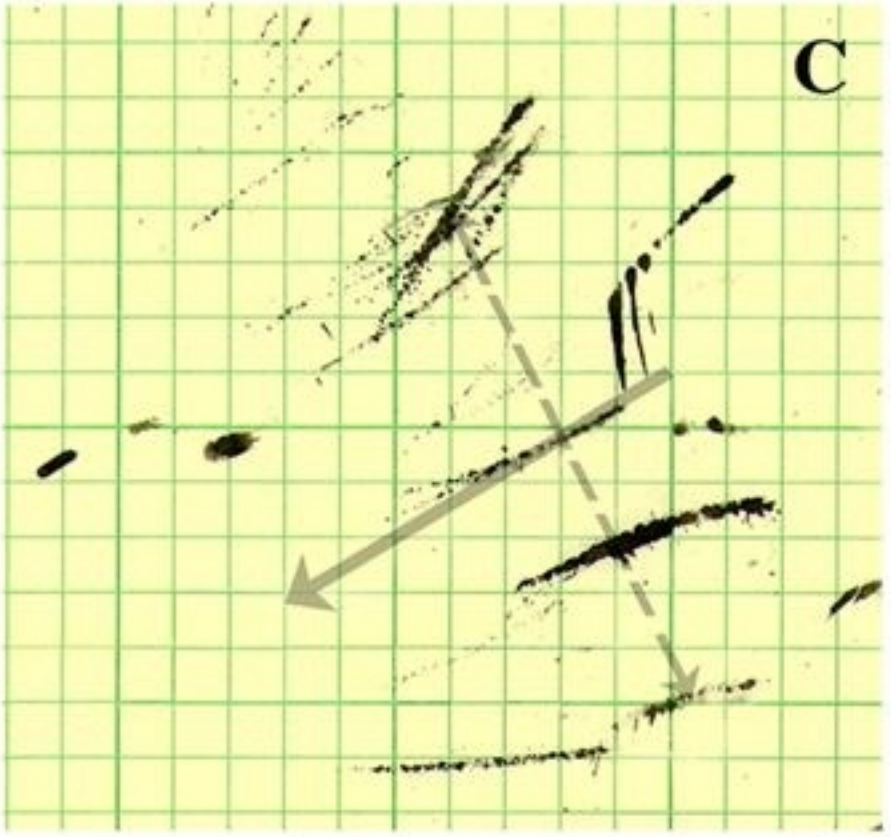
Diseases of oligodendroglia

1st try a cell autonomous defect

e.g., *shiverer* mouse
(MBP-deficient → dysmyelination)

Neural Stem Cells "Complement" The *Shiverer* Mouse By Supplying MBP-expressing Myelinating Oligodendrocytes





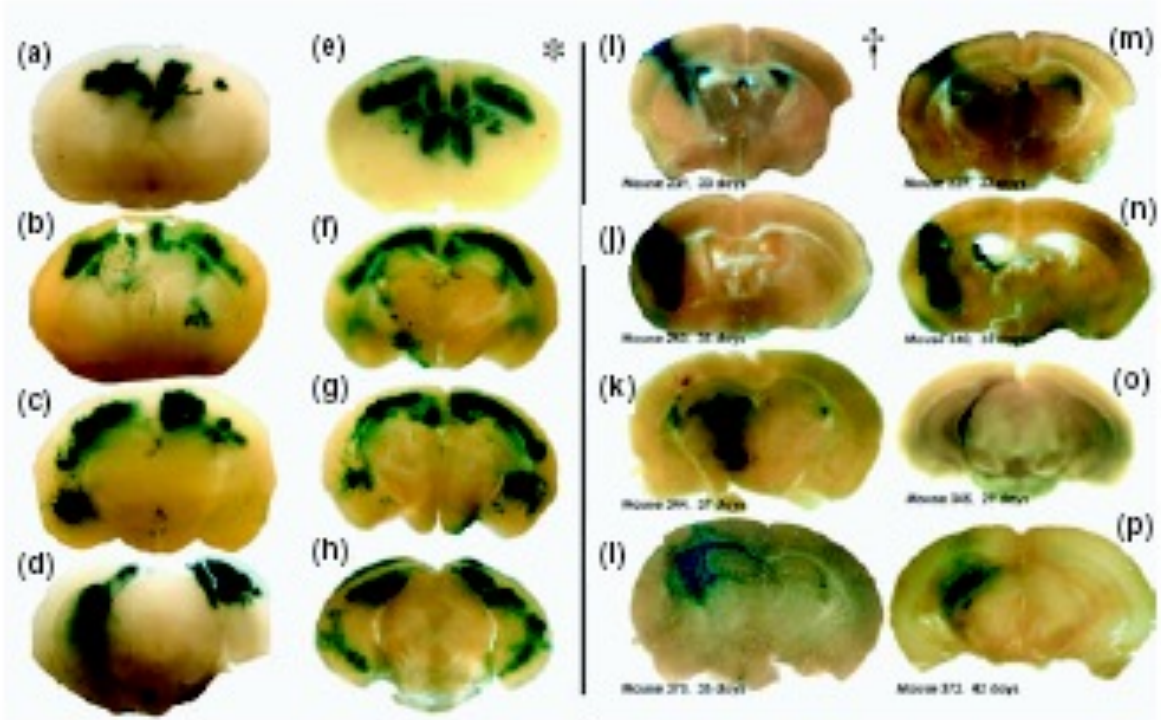
Total body tremor (B, D) prevented and/or eliminated

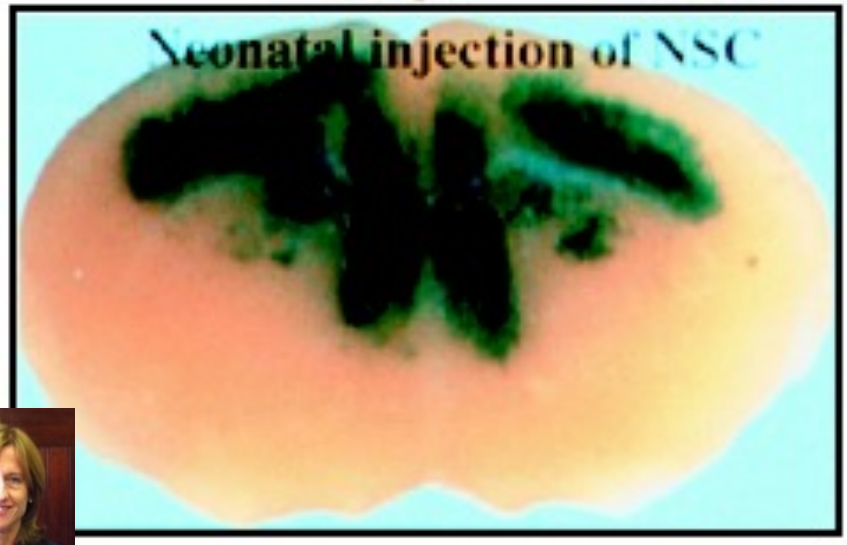
Yandava et al, *PNAS* (1999)

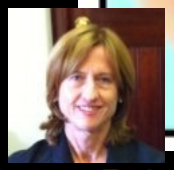
What about a cell *non*-autonomous dys/de-myelination?

e.g., *twitcher* mouse of Krabbe (Globoid Cell) Leukodystrophy
(Galactocerebrosidase [GalC] deficiency ➔ psychosine toxicity)

Twitcher mouse model of Krabbe Disease (GalC deficiency)



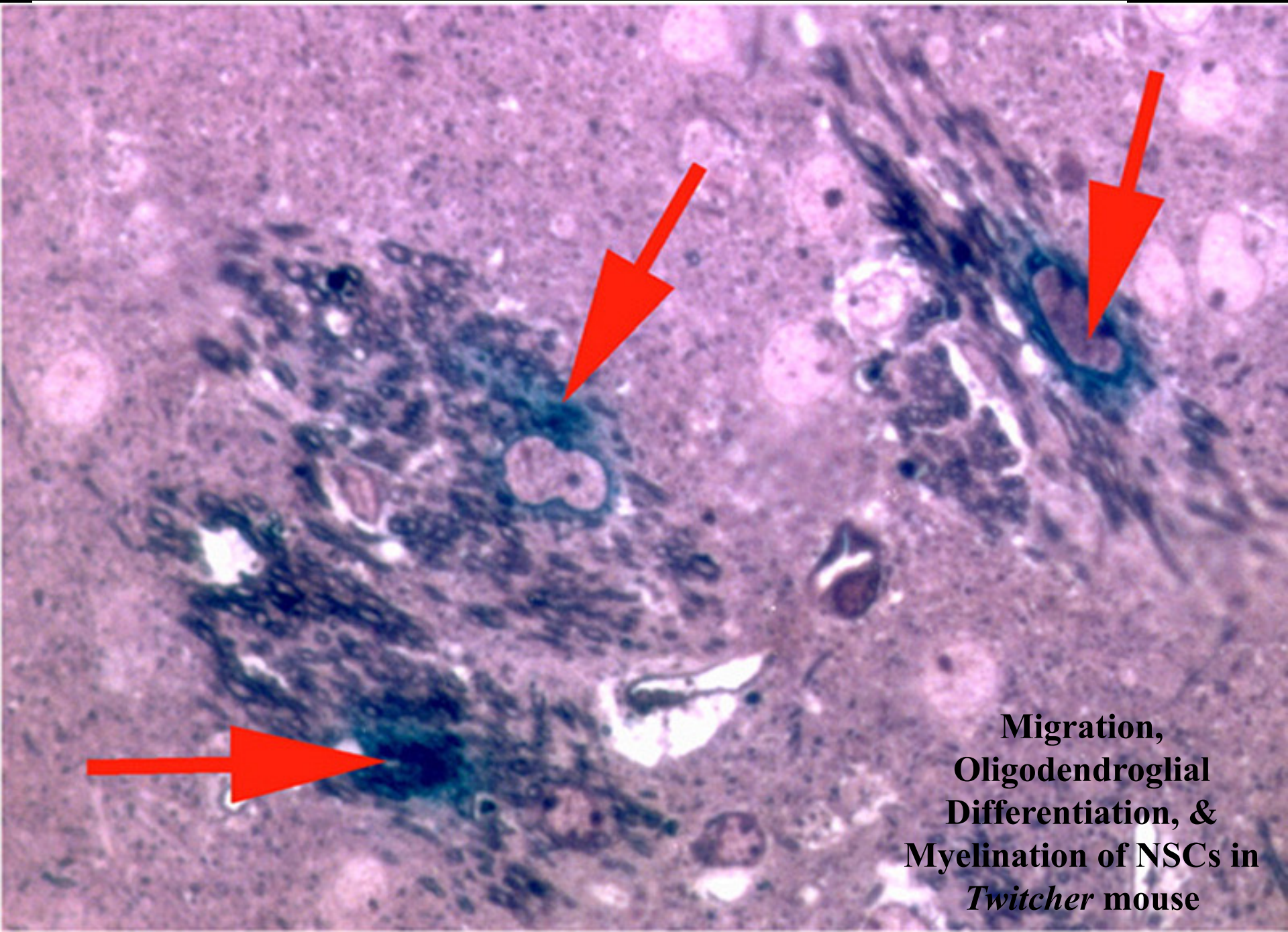




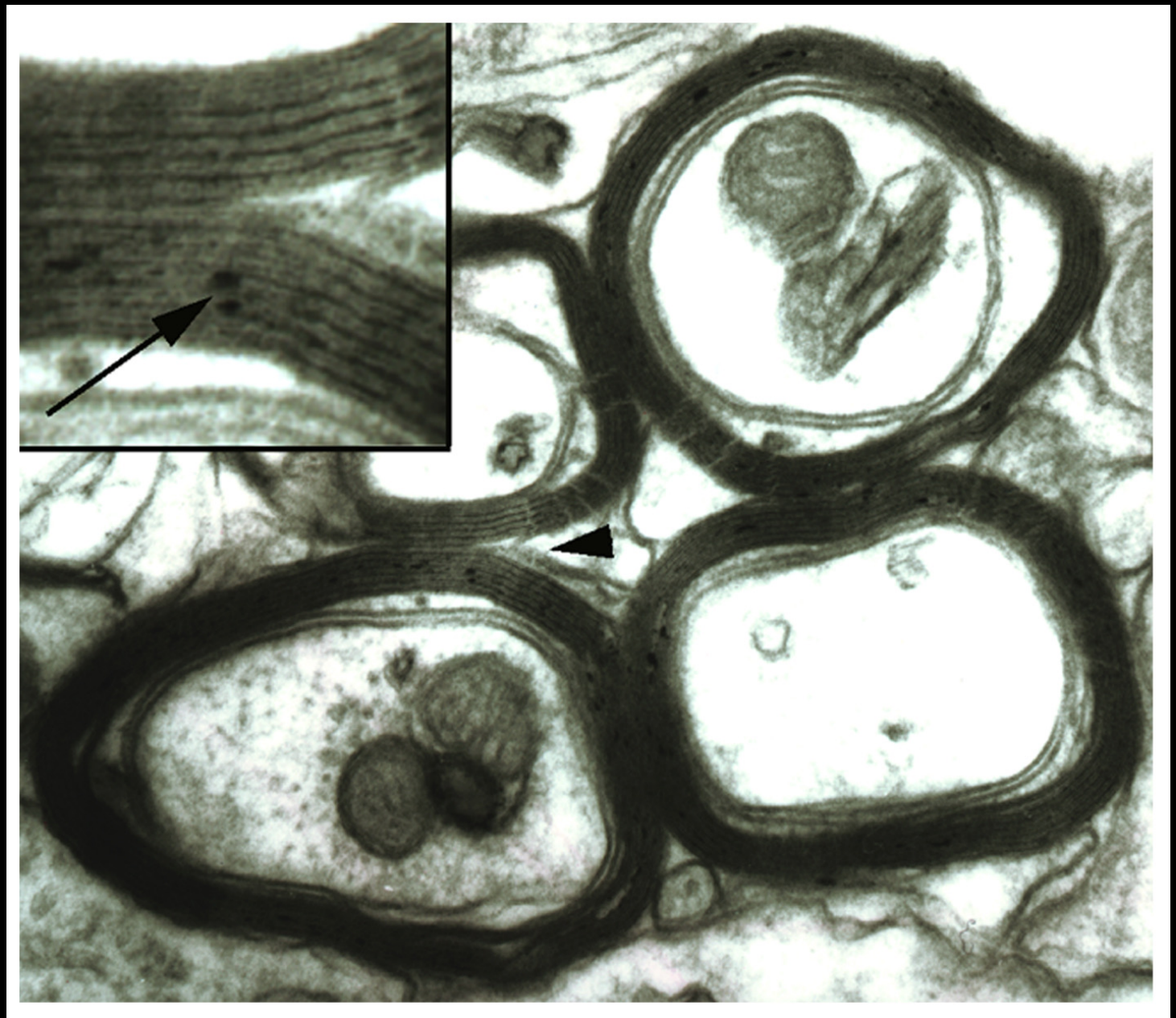
Rosanne Taylor

Xgal (blue) cells = donor NSC-derived cells

Taylor et al, *J Neurochem*, 2006

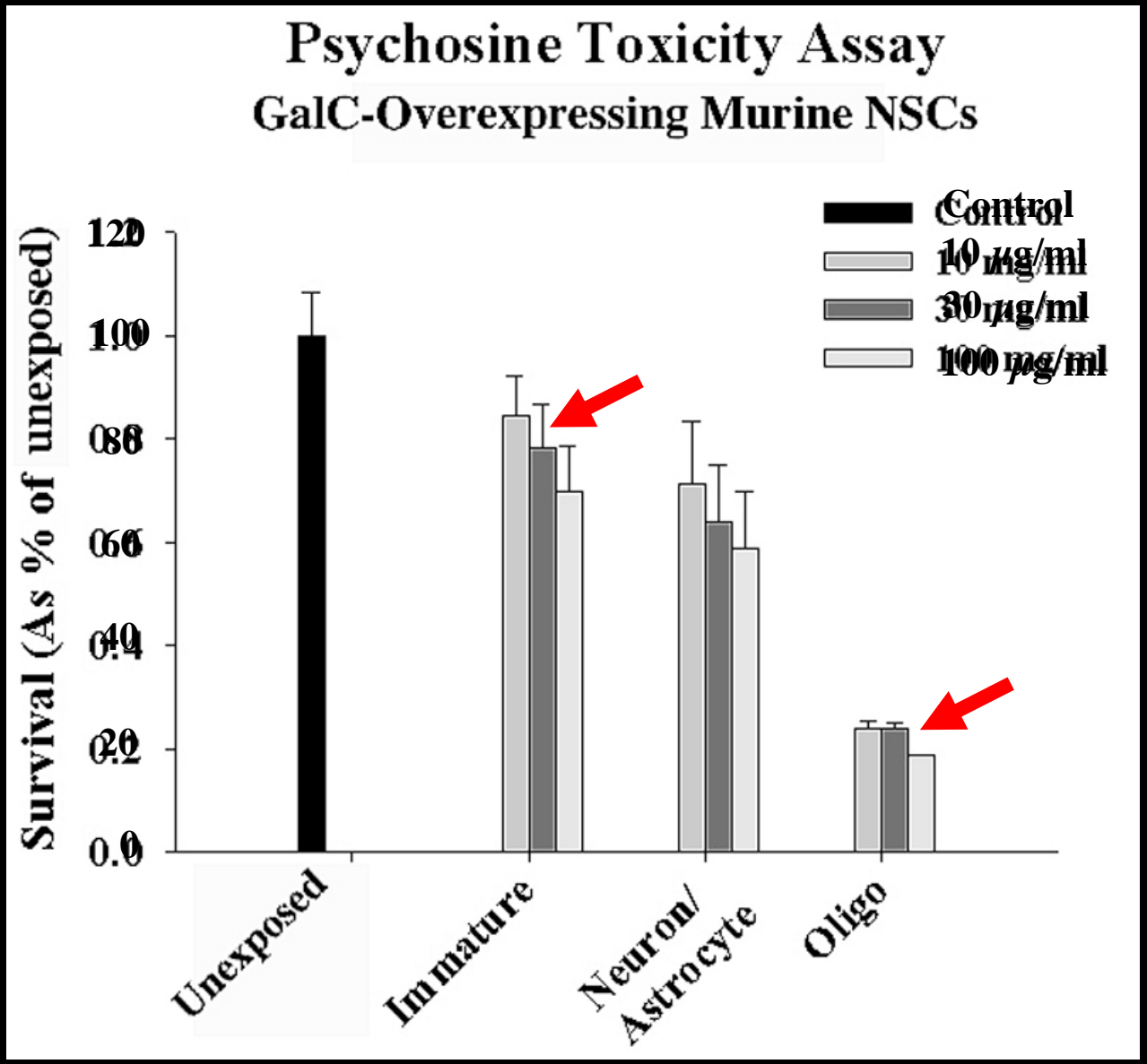

Migration,
Oligodendroglial
Differentiation, &
Myelination of NSCs in
Twitcher mouse

Donor NSC-derived myelin *(note Xgal precipiate [arrow])* in *Twitcher* mouse model of Krabbe Disease



Undifferentiated NSCs most resistant to psychosine while pre-differentiated oligodendrocytes most vulnerable

Growing appreciation, counter-intuitively perhaps, that the more immature a cell, the more resistant, not sensitive, they may be to various stresses (e.g., oxidative, excitotoxic, glutamatergic)



Journal of Neurochemistry, 2006, **97**, 1585–1599

Intrinsic resistance of neural stem cells to toxic metabolites may make them well suited for cell non-autonomous disorders: evidence from a mouse model of Krabbe leukodystrophy

Roseanne M. Taylor,* Jean Pyo Lee,†‡ James J. Palacino,‡ Kate A. Bower,‡ Jianxue Li,‡ Marie T. Vanier,§ David A. Wenger,¶ Richard L. Sidman‡ and Evan Y. Snyder†‡

Taylor et al, *J Neurochem*, 2006

LESSON:

Neural cell replacement via NSCs may be feasible

if

- Defect Intrinsic to Host Cell
- Donor Cells are *"Resistant"* to a Defect Extrinsic to the Host Cell
  - Inherently or engineered to be so
  - Resistance/Sensitivity sometimes dependent on differentiation state of NSC & that of cells surrounding them

Must try to know mechanism of pathological action of a given disease because will influence feasibility of approach:

- Cell autonomous (intrinsic)?
- Cell non-autonomous (extrinsic)?
- Mixed?

Must try to know range of homeostatic mechanisms to invoke

May be neither
- diffusible factor or
- cell replacement
- but rather cell-cell contact

Gap junction formation via *connexins* are a normal mechanism for intercellular communication during development

(especially Cx43)

PNAS

Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host

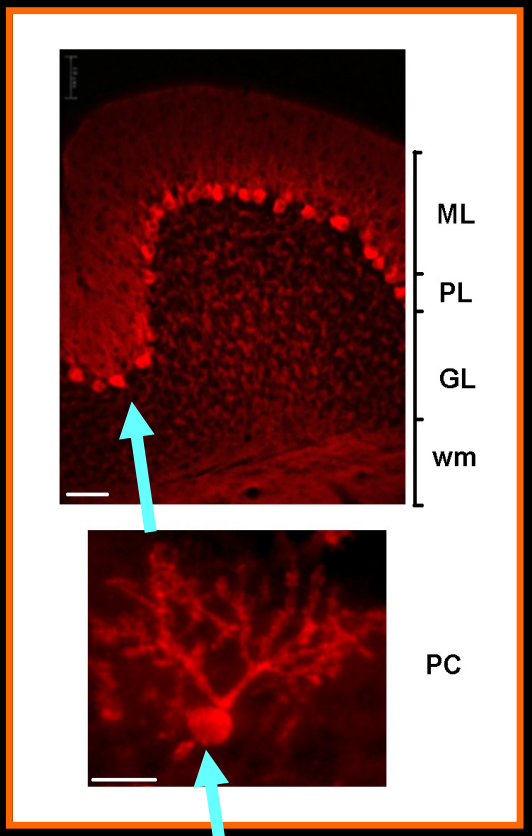
Johan Jäderstad[a,1], Linda M. Jäderstad[a,1], Jianxue Li[b,2], Satyan Chintawar[c,2], Carmen Salto[d], Massimo Pandolfo[c], Vaclav Ourednik[b], Yang D. Teng[e], Richard L. Sidman[b,3], Ernest Arenas[d], Evan Y. Snyder[b,f,3], and Eric Herlenius[a,3]

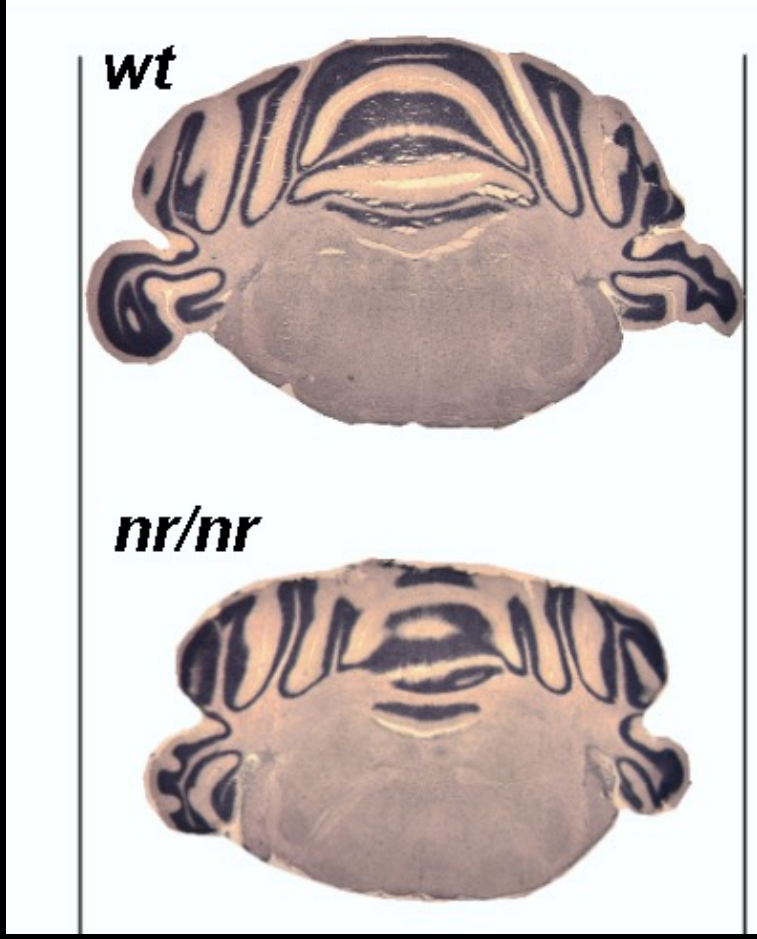
[a]Department of Women's and Children's Health and; [d]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 17176 Stockholm, Sweden; [b]Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA 02215; [c]Service de Neurologie, Hôpital Erasme–University Libre de Bruxelles, 1070 Brussels, Belgium; [e]Department of Neurosurgery, Brigham & Women's Hospital, Boston, MA 02215; and [f]Burnham Institute for Medical Research, La Jolla, CA 92037

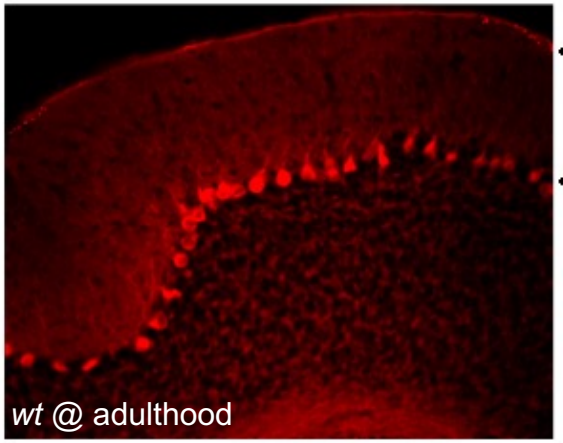
5184–5189 | PNAS | March 16, 2010 | vol. 107 | no. 11

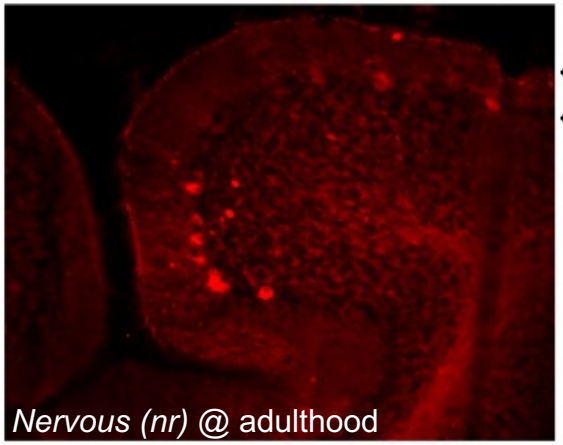
www.pnas.org/cgi/doi/10.1073/pnas.0915134107

Eric Herlenius







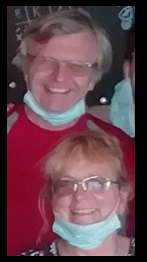


0.5 cm

50 µm

Cerebellar Purkinje Cell Neuron Degeneration Mutants (*"nervous"*)

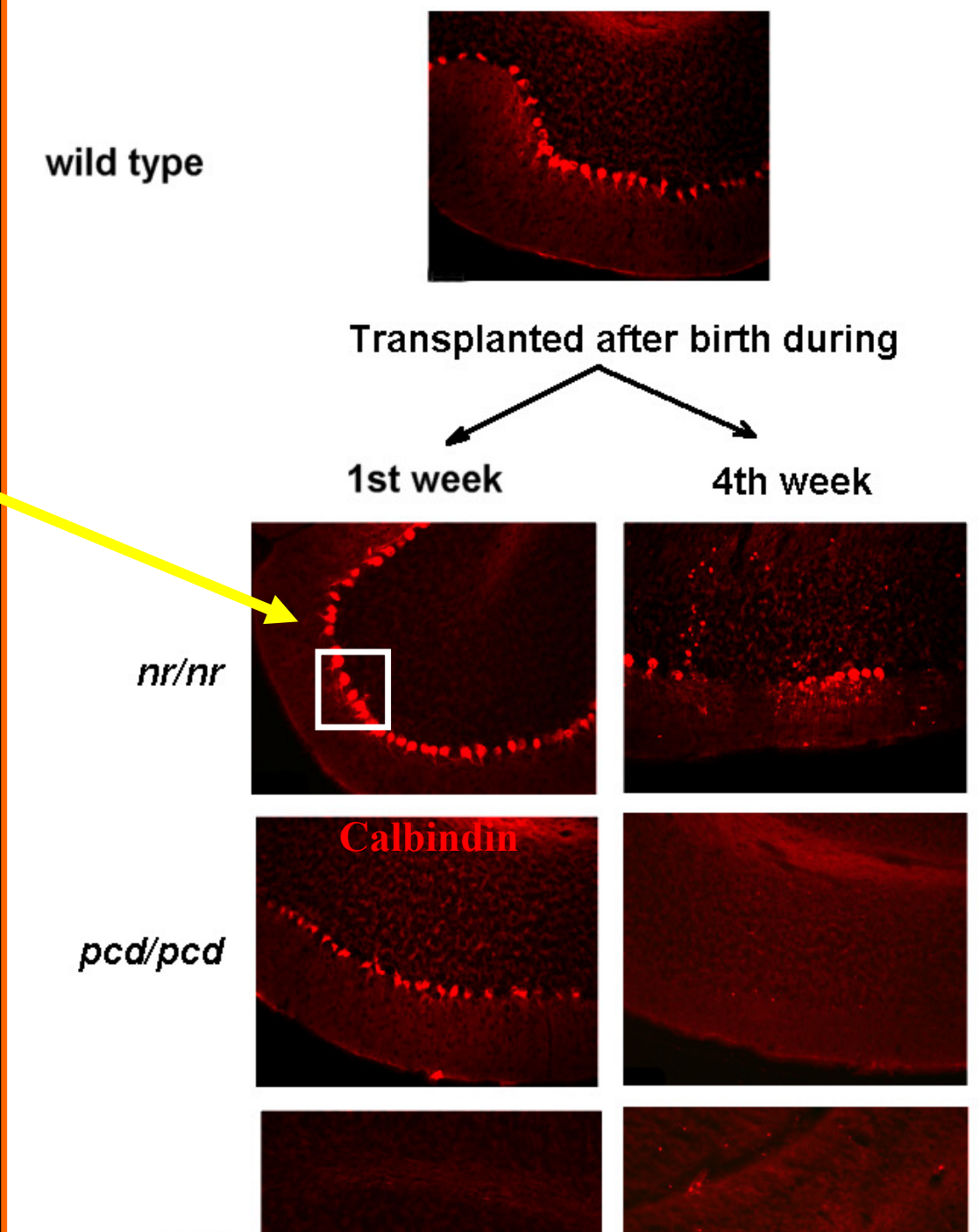
Dick Sidman



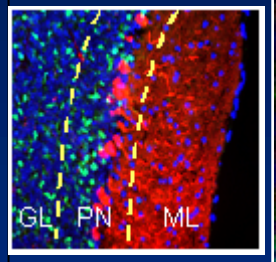
Vaclav & Jitka Ourednik

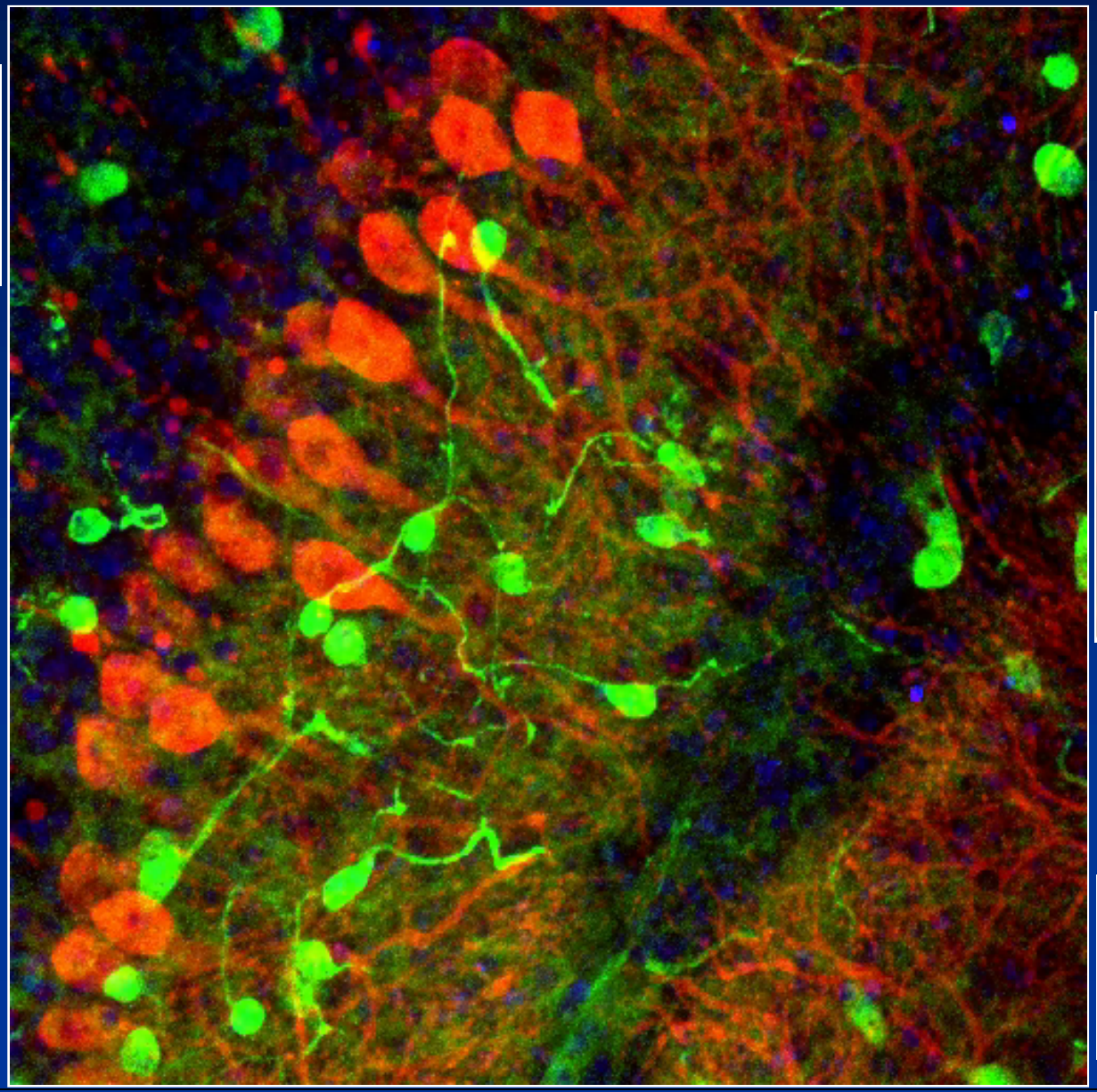
Li et al, *PNAS* & *J Neurosci* 2006

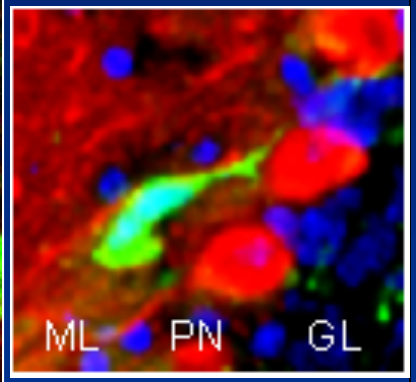
Purkinje Cell Layer developed & persisted following transplantation of neural stem cells at birth



Rescue of adult mutant *Nervous* Purkinje Neurons by neonatally-transplanted donor NSCs that make cell-cell contact & form gap junctions with them (re-equilibrating their disordered metabolism)



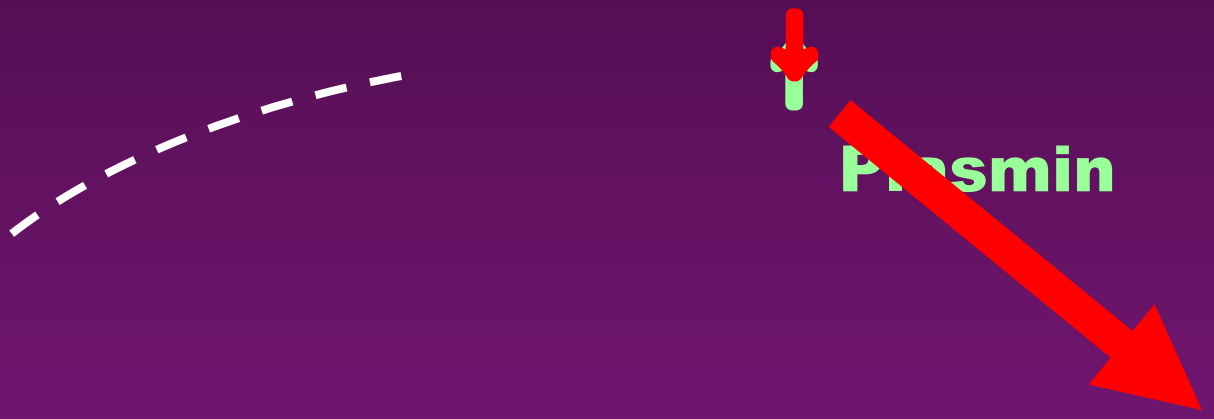




Jaderstad et al, *PNAS* (2010)

Li, et al, *PNAS*; *J. Neurosci.* (2006)

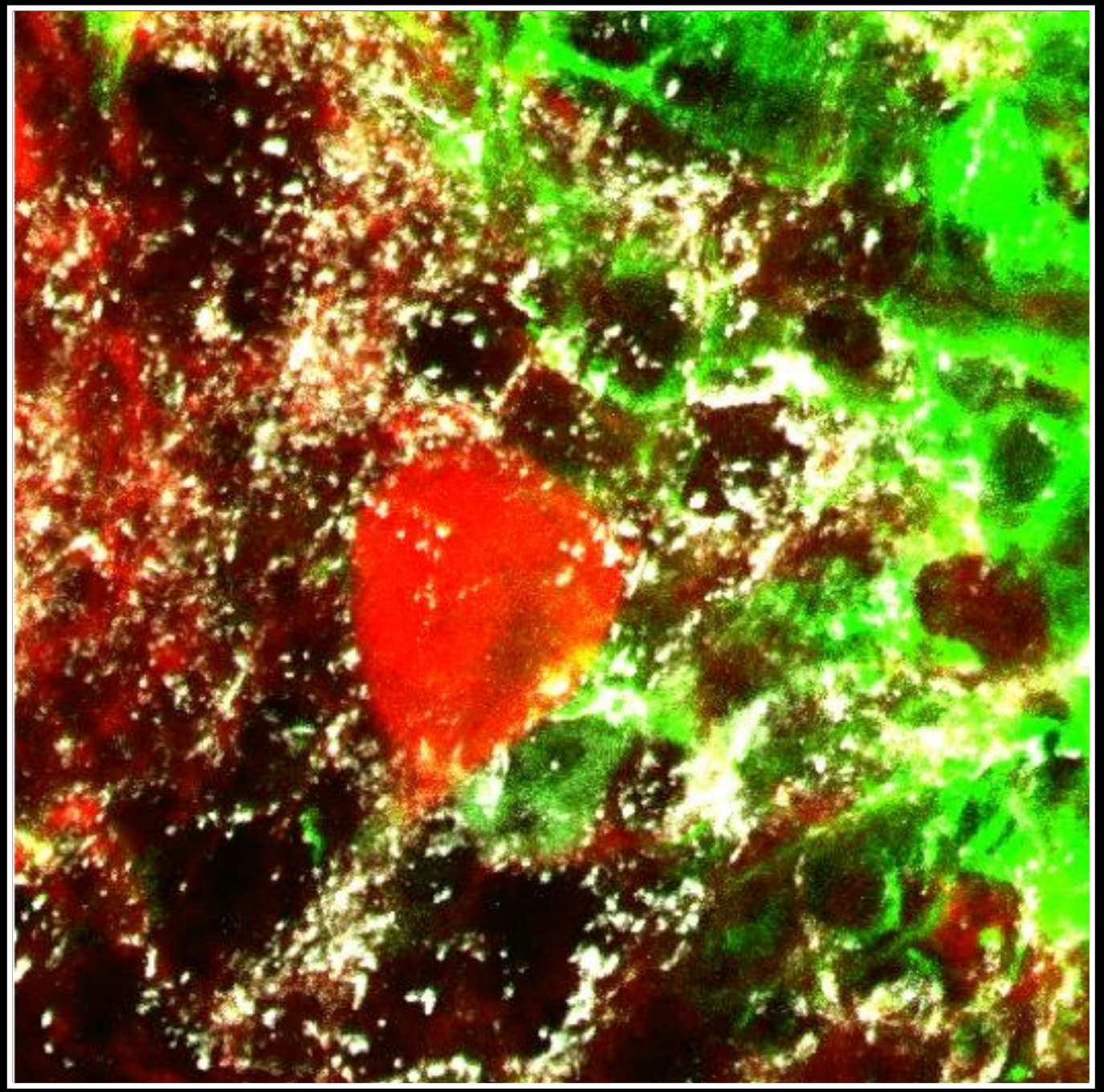
βgal (NSC)
Calb (PN)
Dapi (nuclei)



More Normal Motor Behavior

Li, et al, *PNAS*; *J. Neurosci*. (2006)

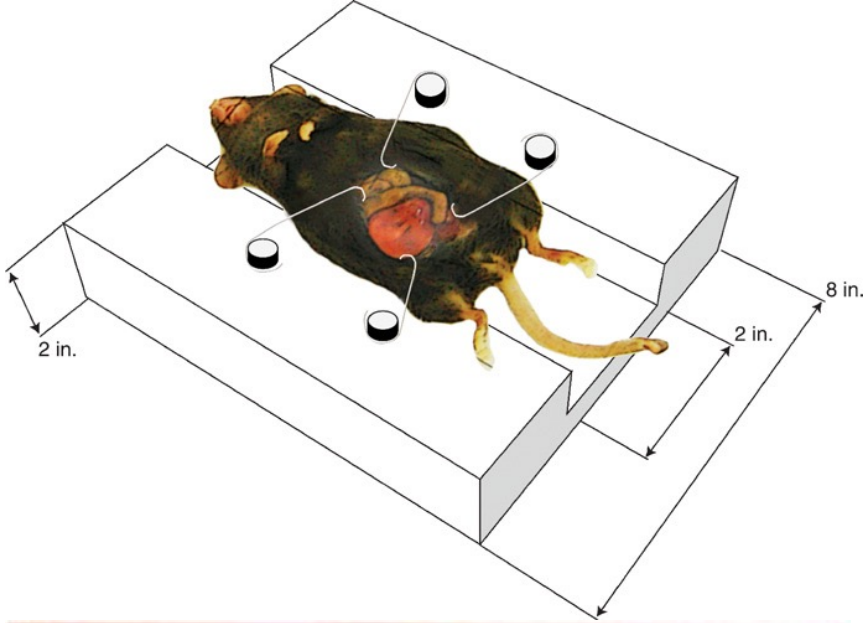
Cx43
(gap junctions)
on SCA1
Purkinje Neurons
(soma & dendrites)
emanating from
grafted NSCs

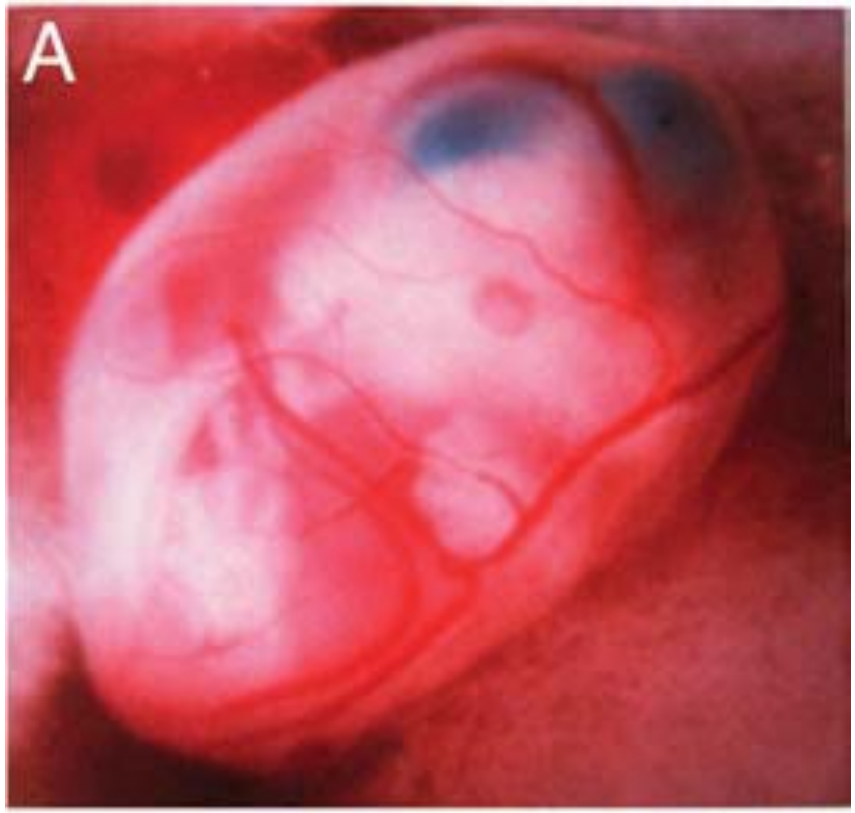


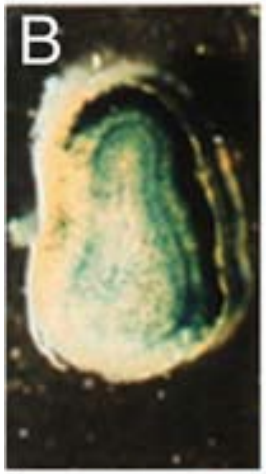
Jaderstad et al, *PNAS* (2010)

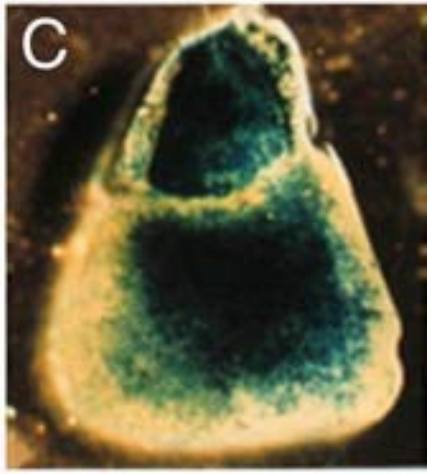
What about treating neurological problems *extremely* early, *before* there are symptoms (e.g., during cerebrogenesis) by integrating normal cells among abnormal cells?

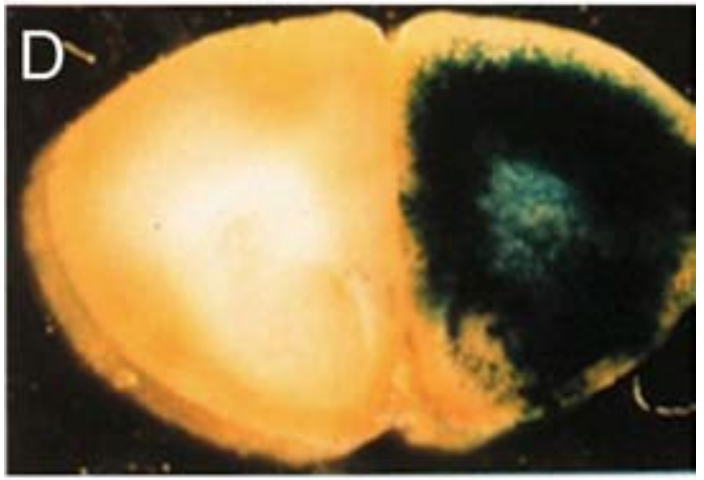
1st: Do the cells have the capacity to participate in normal cerebrogenesis?

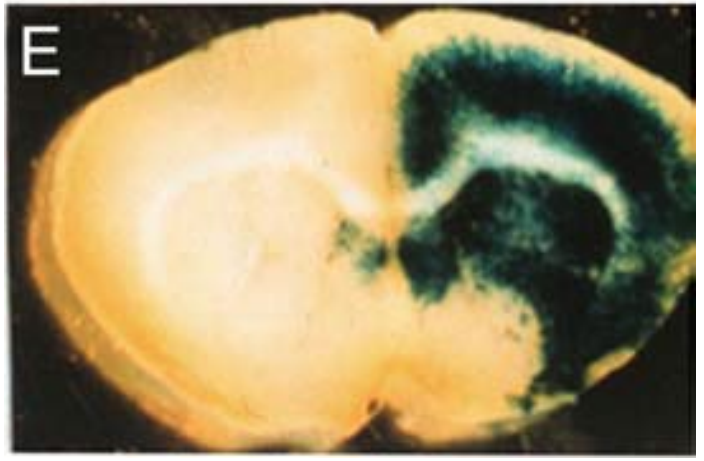




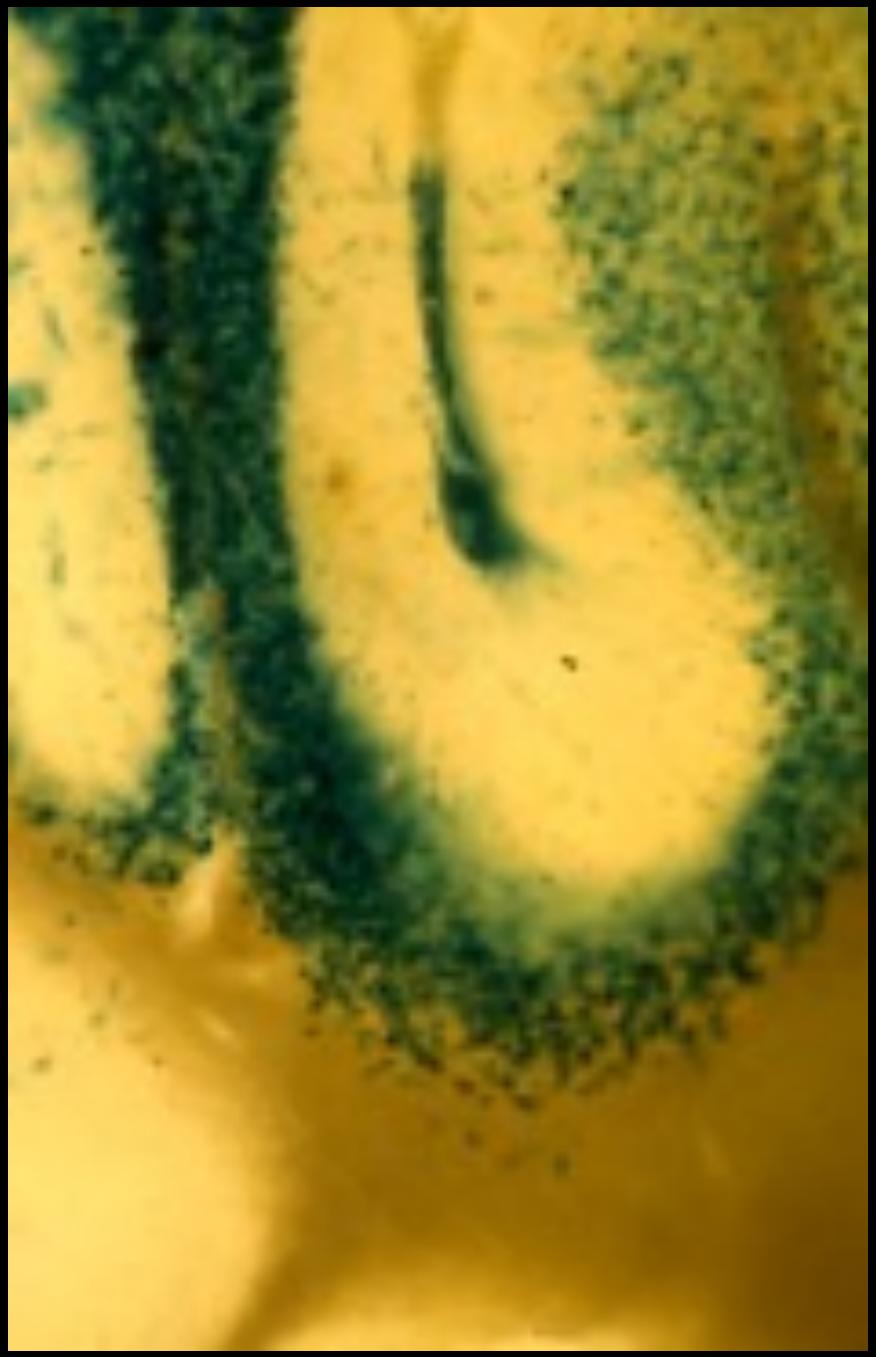


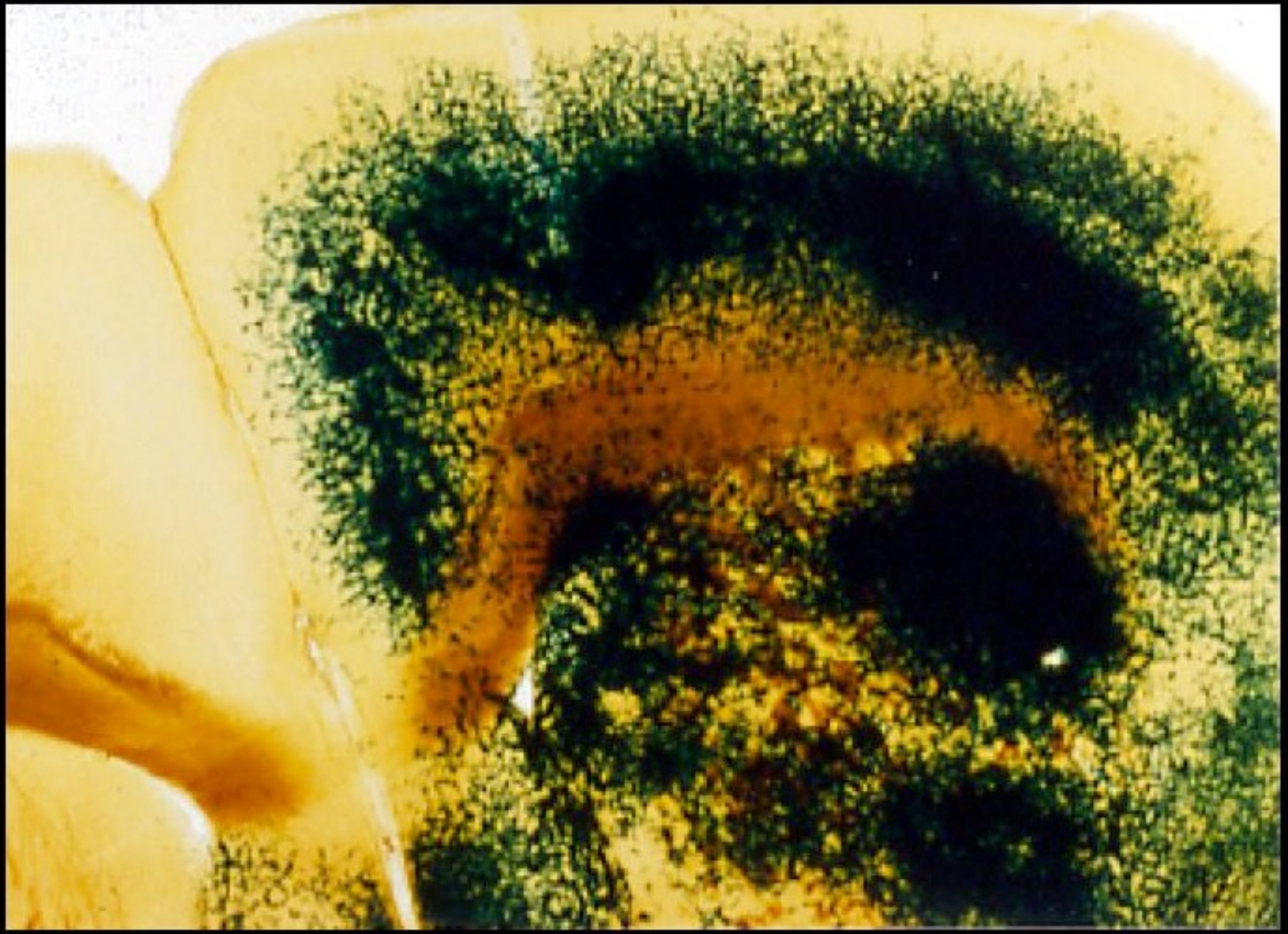


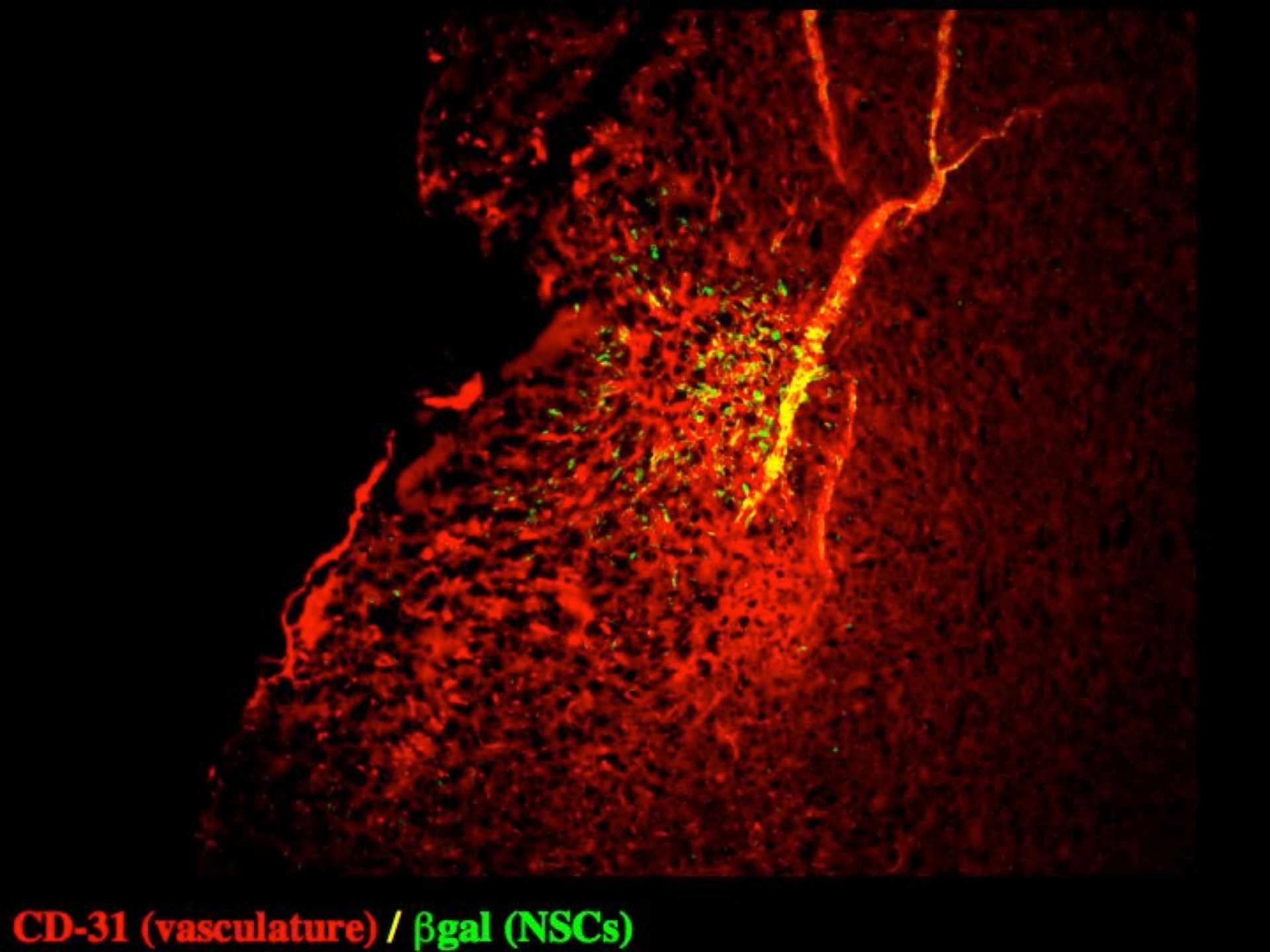


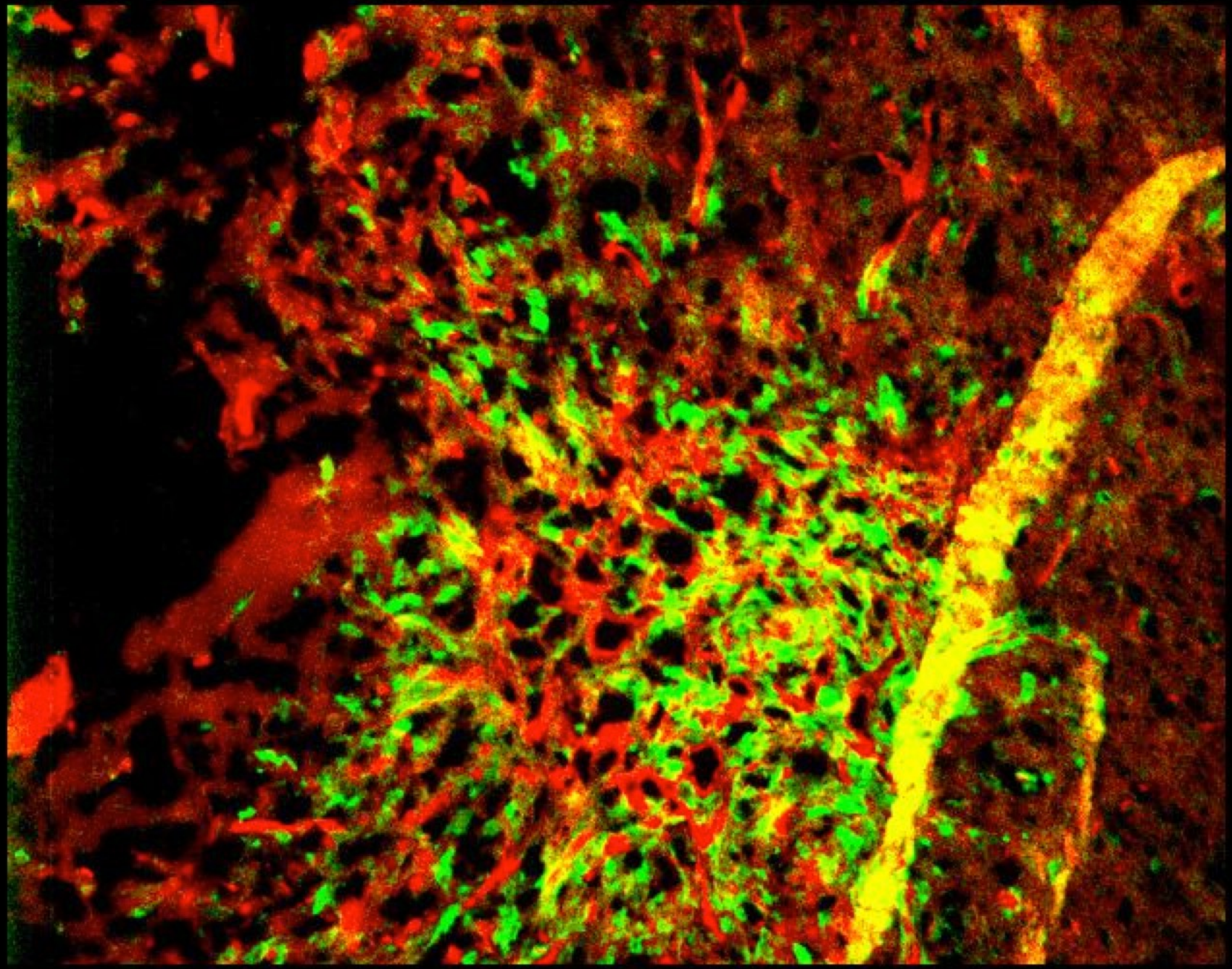


- Park et al, *Exp Neurol* (2006)
- Lee J-P et al, *Curr Protoc Neurosci* (2008)

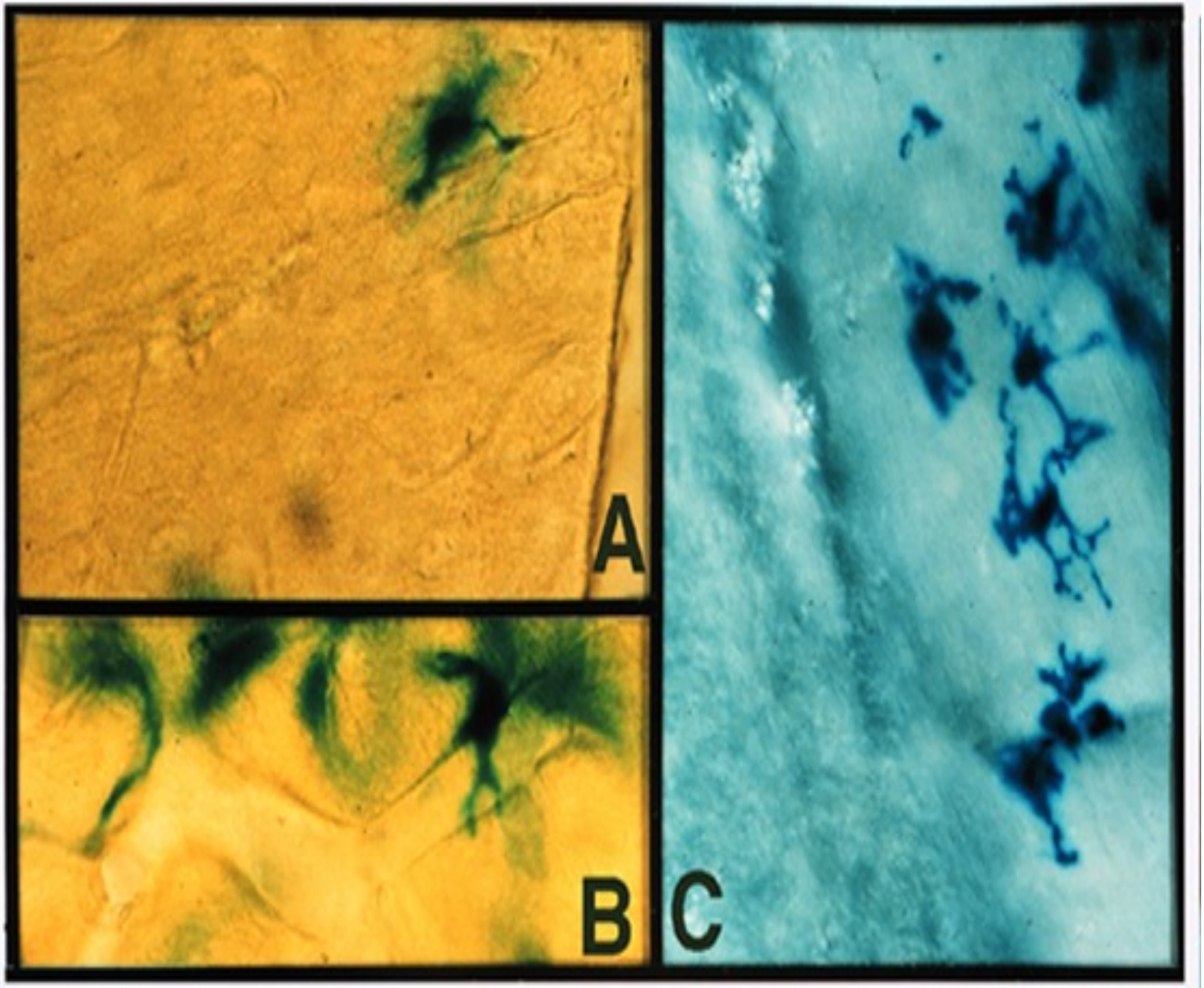


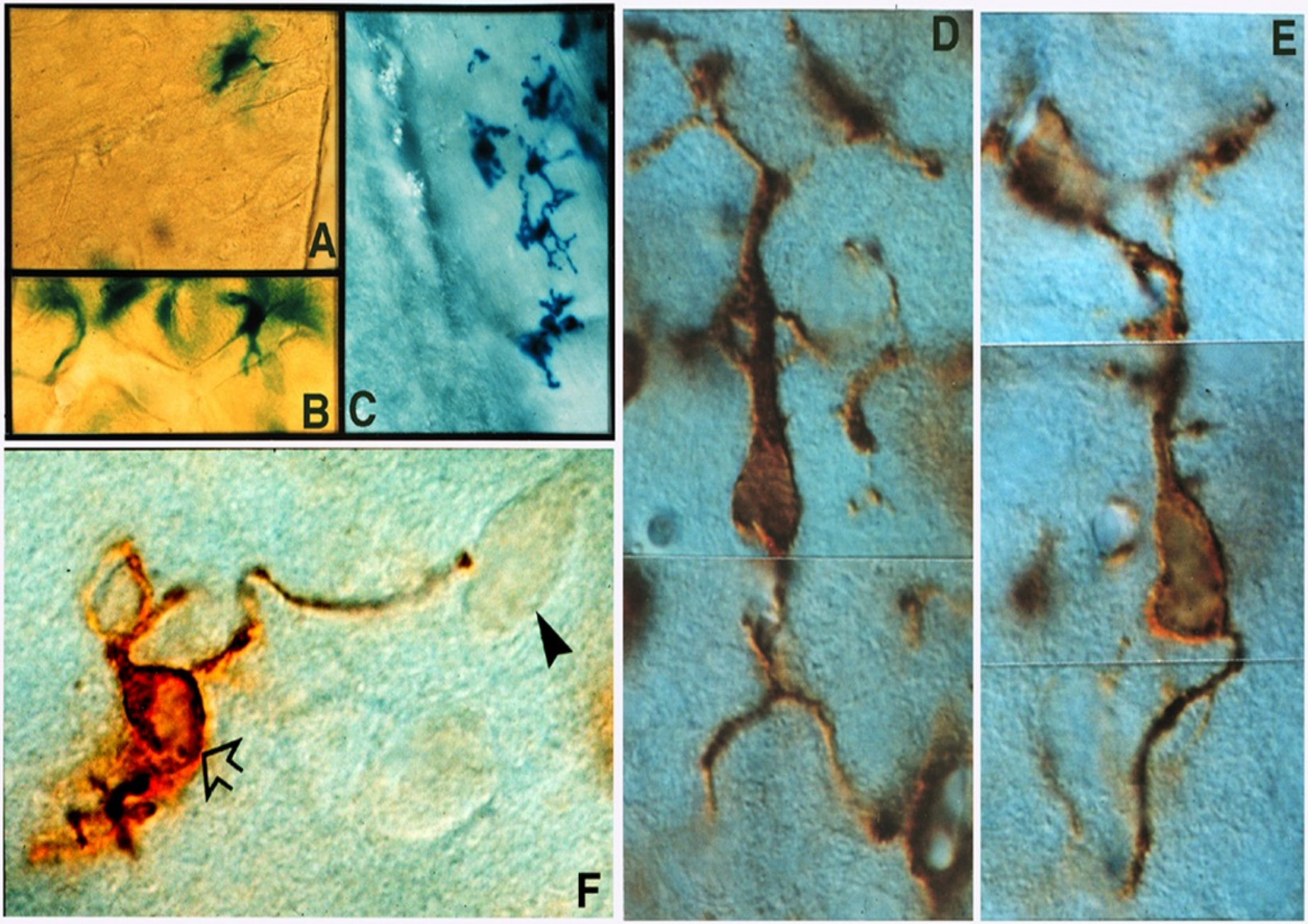



CD-31 (vasculature) / βgal (NSCs)



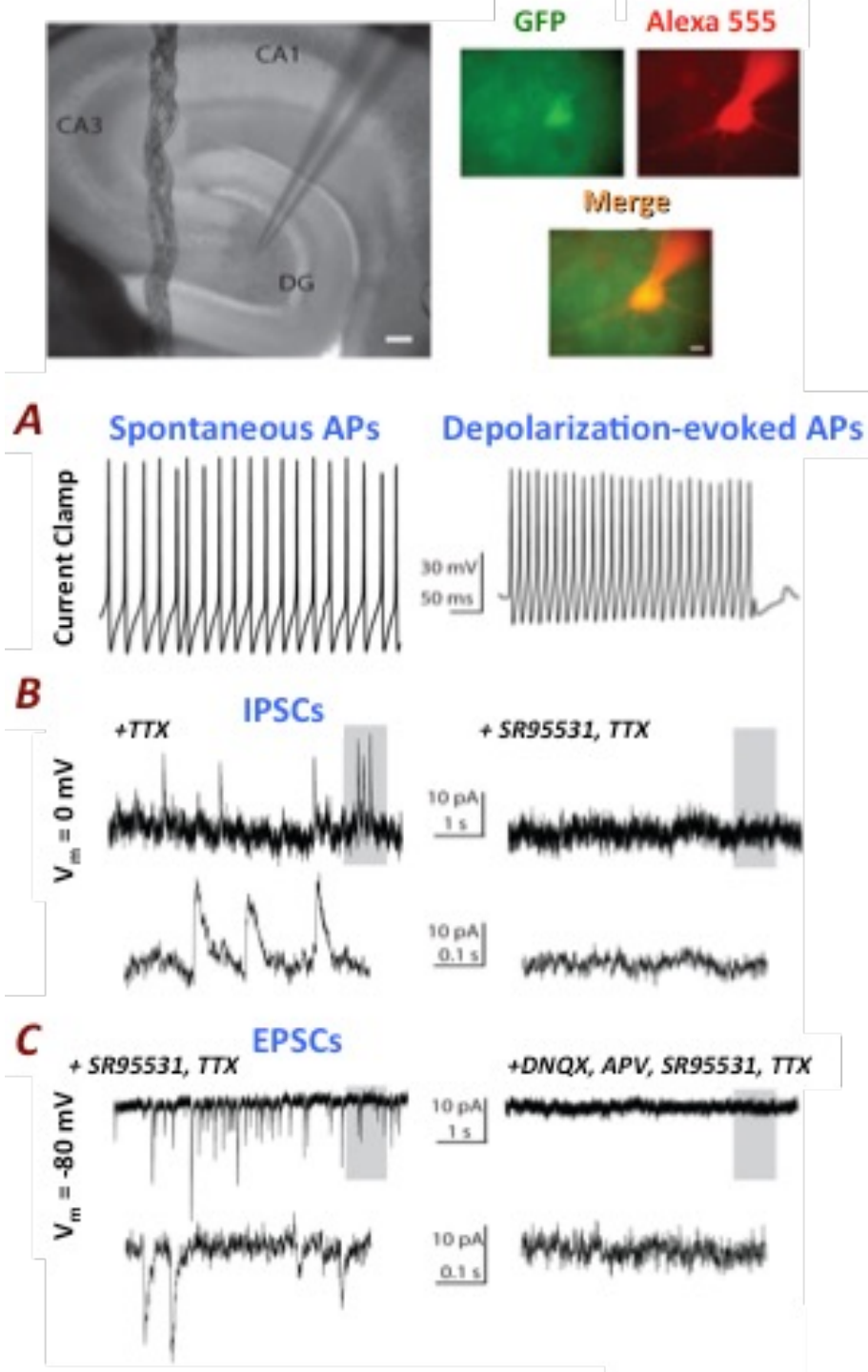
CD-31 (vasculature) / βgal (NSCs)


A
B
C

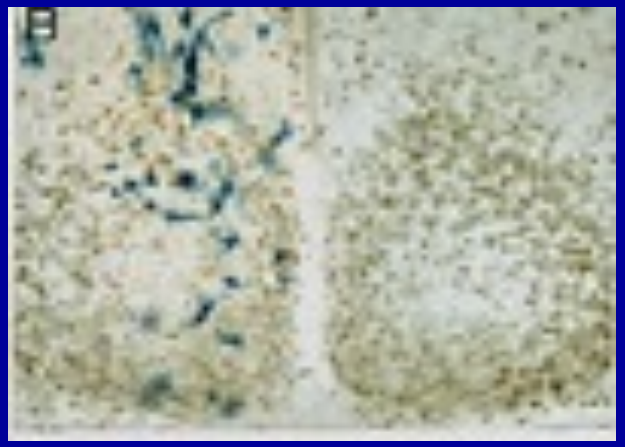

A
B
C
D
E
F

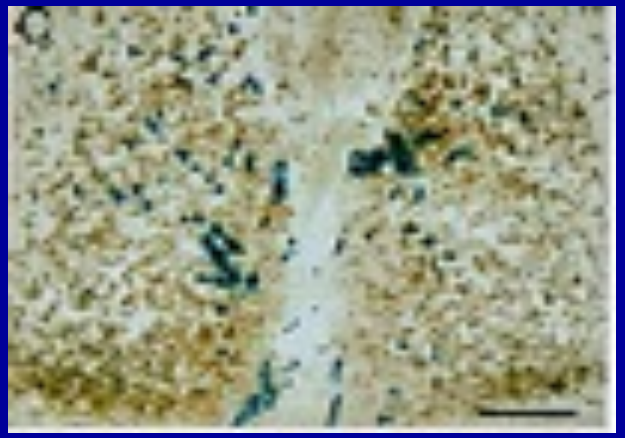

d
PN
PN
A
d
Ip
A

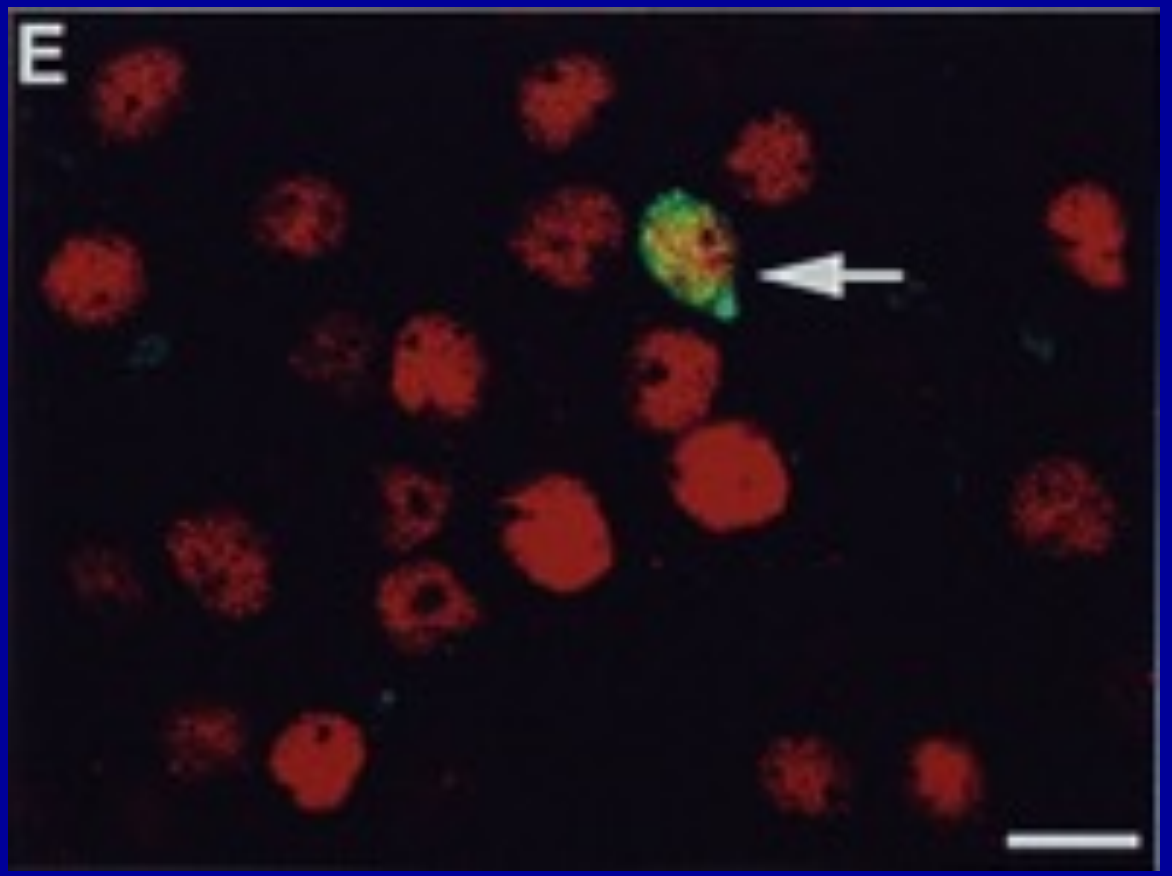
hNSCs functionally integrated into cortex of mouse brain following in utero transplantation (spiking action potentials)



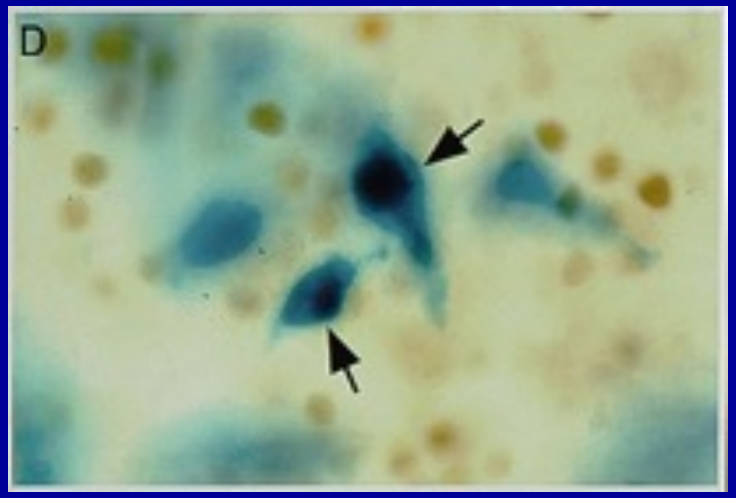
J-P Lee et al, PNAS 2020







c-fos / ßgal



c-fos / ßgal

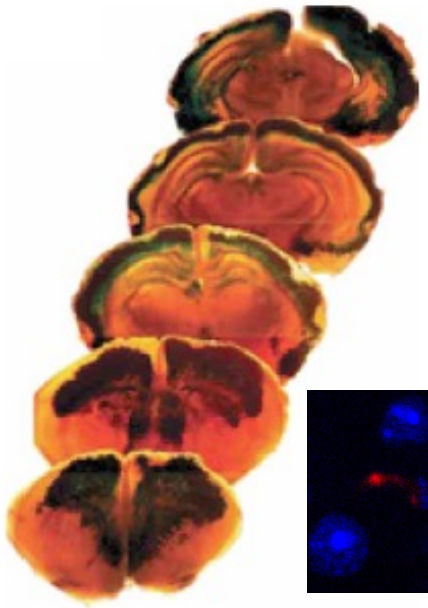
Bill Schwartz

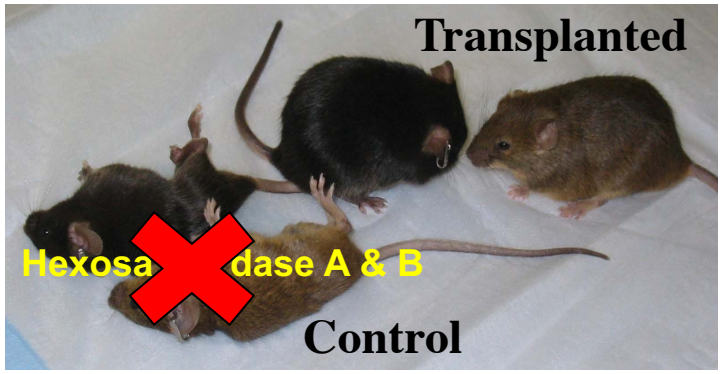
Sandhoff Disease (Hexosaminidase B deficiency)

nature medicine

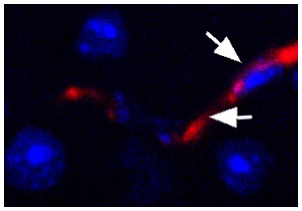
Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease

Jean-Pyo Lee[1,2,12], Mylvaganam Jeyakumar[3,12], Rodolfo Gonzalez[1], Hiroto Takahashi[1,11], Pei-Jen Lee[1], Rena C Baek[4], Dan Clark[1], Heather Rose[1], Gerald Fu[1], Jonathan Clarke[1], Scott McKercher[1], Jennifer Meerloo[1], Franz-Josef Muller[1,5], Kook In Park[6], Terry D Butters[3], Raymond A Dwek[3], Philip Schwartz[7], Gang Tong[1,8], David Wenger[9], Stuart A Lipton[1,8], Thomas N Seyfried[4], Frances M Platt[3] & Evan Y Snyder[1,2,10]

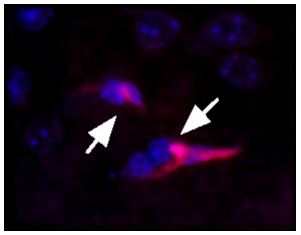




NSCs Impact Sandhoff Disease



1° human NSCs

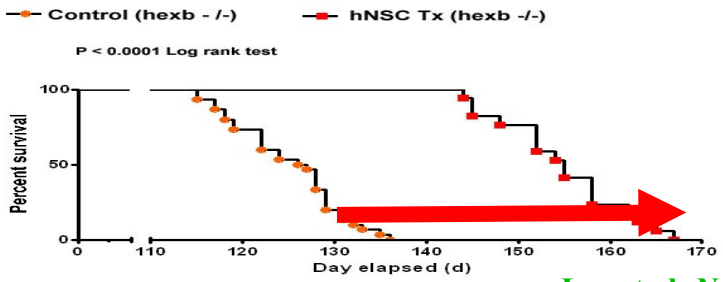


2° human NSCs (hESC-derived NSCs)

Rotarod

B.

Lifespan



Lee et al, *Nature Med* (2007)

Mechanisms of Action of the NSCs

- Enzyme Replacement
- Reduction in GM2 lysosomal storage
- Restoration of normal cellular metabolism
- Restoration of normal lysosomal function
- Anti-inflammation
- Trophic &/or Neuroprotective support
- ? Neural cell replacement – *maybe, if concept of "cell replacement" broadened to think beyond "neurons"*

Must always be aware that…

…stem cells will follow their normal biologically-determined differentiation programs & imperatives…..

Experimental Neurology 230 (2011) 75–77

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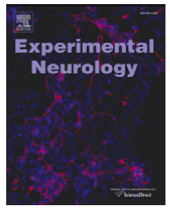
journal homepage: www.elsevier.com/locate/yexnr



Commentary

The risk of putting something where it does not belong: Mesenchymal stem cells produce masses in the brain

Evan Y. Snyder

Variable behavior and complications of autologous bone marrow mesenchymal stem cells transplanted in experimental autoimmune encephalomyelitis

Nikolaos Grigoriadis [a,*], Athanasios Lourbopoulos [a], Roza Lagoudaki [a], Josa-Maria Frischer [b], Eleni Polyzoidou [a], Olga Touloumi [a], Constantina Simeonidou [c], Georgia Deretzi [a], Jannis Kountouras [a], Evangelia Spandou [c], Konstantia Kotta [d], Georgios Karkavelas [e], Nikolaos Tascos [a], Hans Lassmann [b]

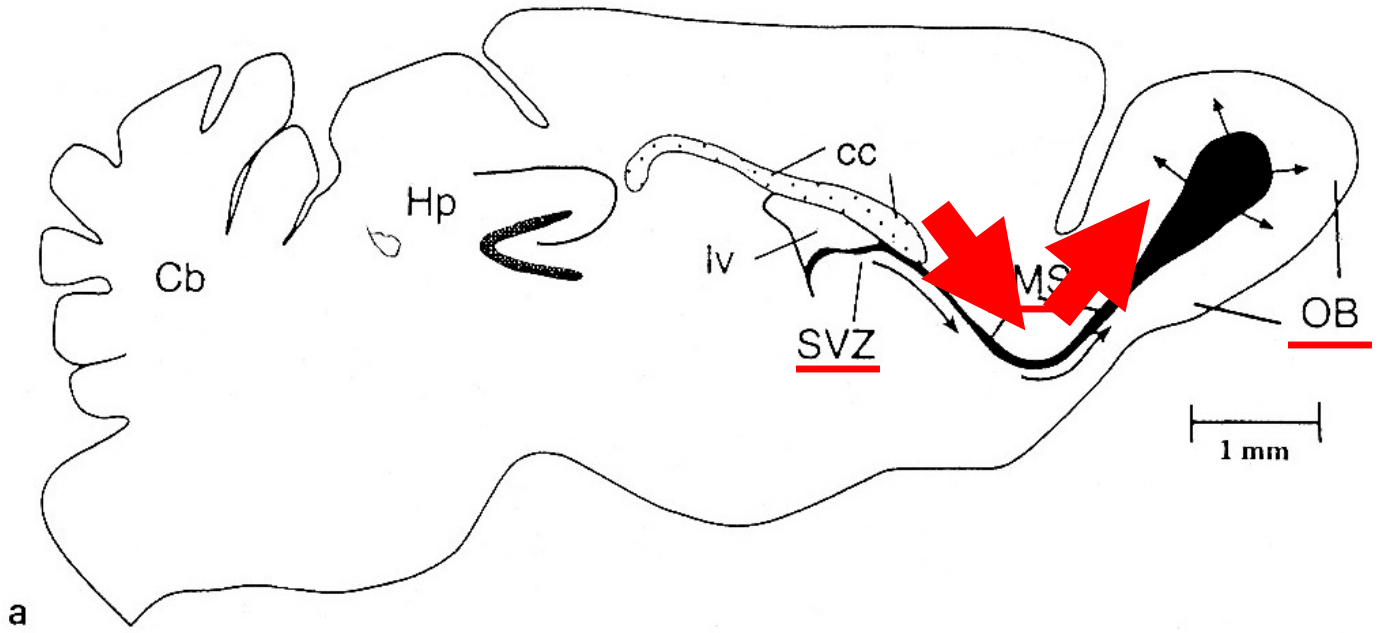
MSCs produced connective-tissue-containing masses in response to the inflammatory cytokines present in brain pathology modeling Multiple Sclerosis; i.e., MSCs simply playing out their normal biology

- Have seen how a proper stem cell should engage in developmental processes, &, if normal, can complement or cross-correct a defect

Test Case:

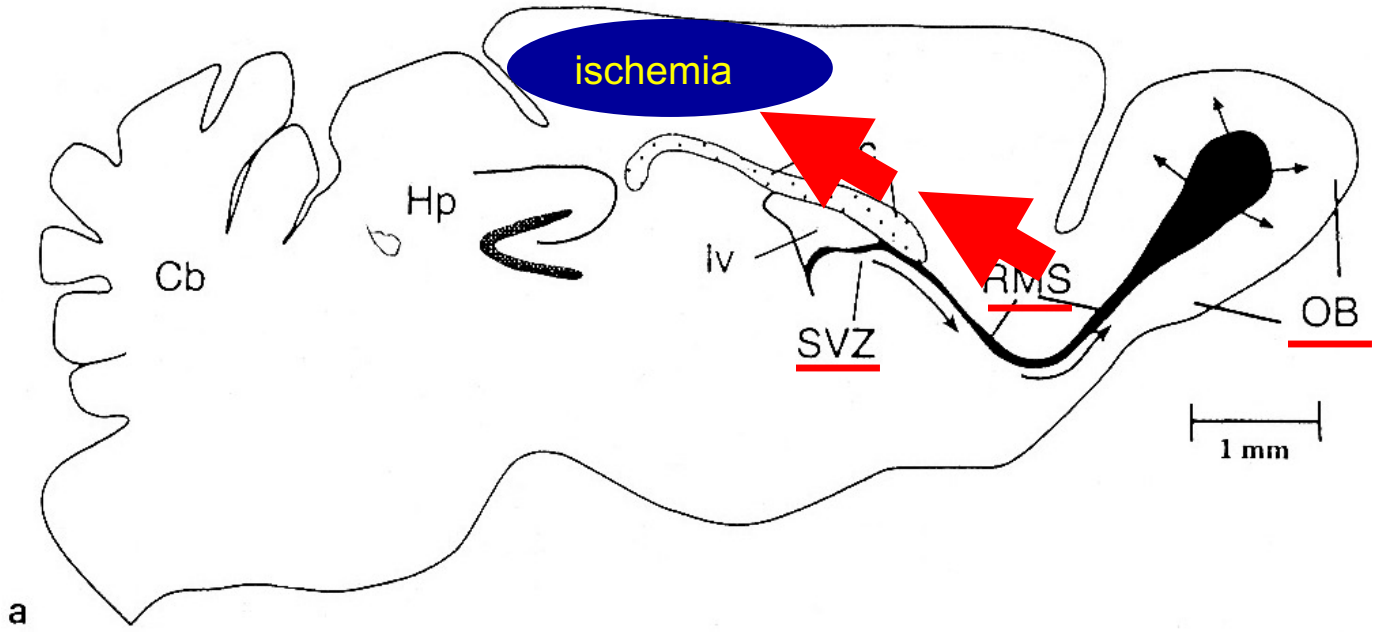
- *Perinatal hypoxic-ischemic injury (HII) (also called "Perinatal Asphyxia")*
  Tissue damage caused by lack of oxygenated blood flow to neonatal organs before, during, or immediately after birth
- In brain ➔ "*Hypoxic Ischemic Encephalopathy (HIE)*"
  Like *"perinatal stroke"*
- *Most common cause of cerebral palsy*

Constitutive, homeostasis-preserving *Developmental "Programs"* *inherently* in place to deal with perturbations in the CNS & to try to reconstitute the system



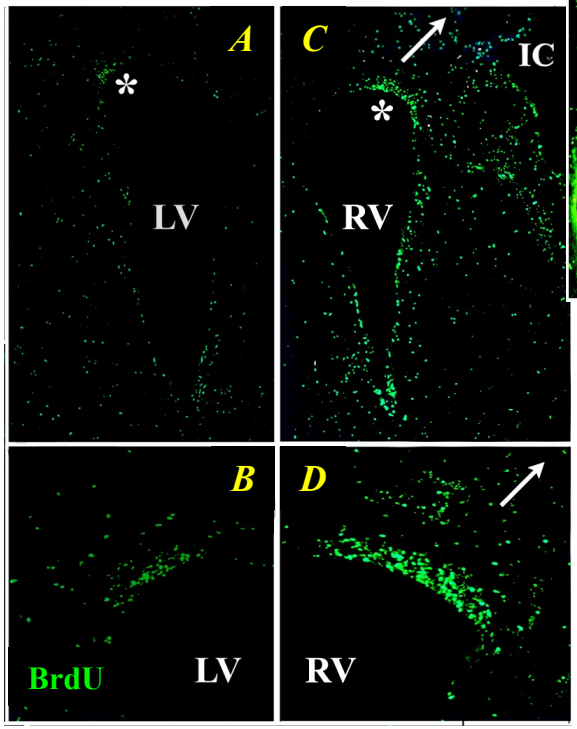
Kook In Park

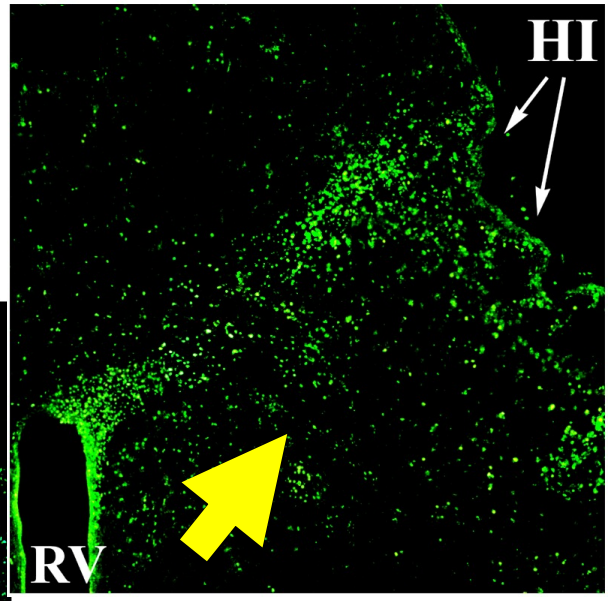
Constitutive, homeostasis-preserving *Developmental "Programs"* *inherently* in place to deal with perturbations in the CNS & to try to reconstitute the system



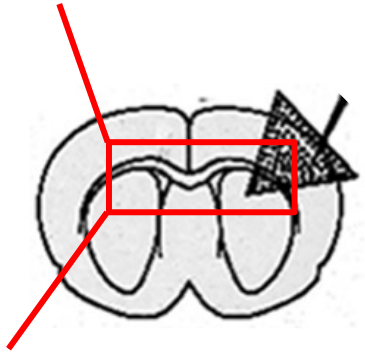
Kook In Park

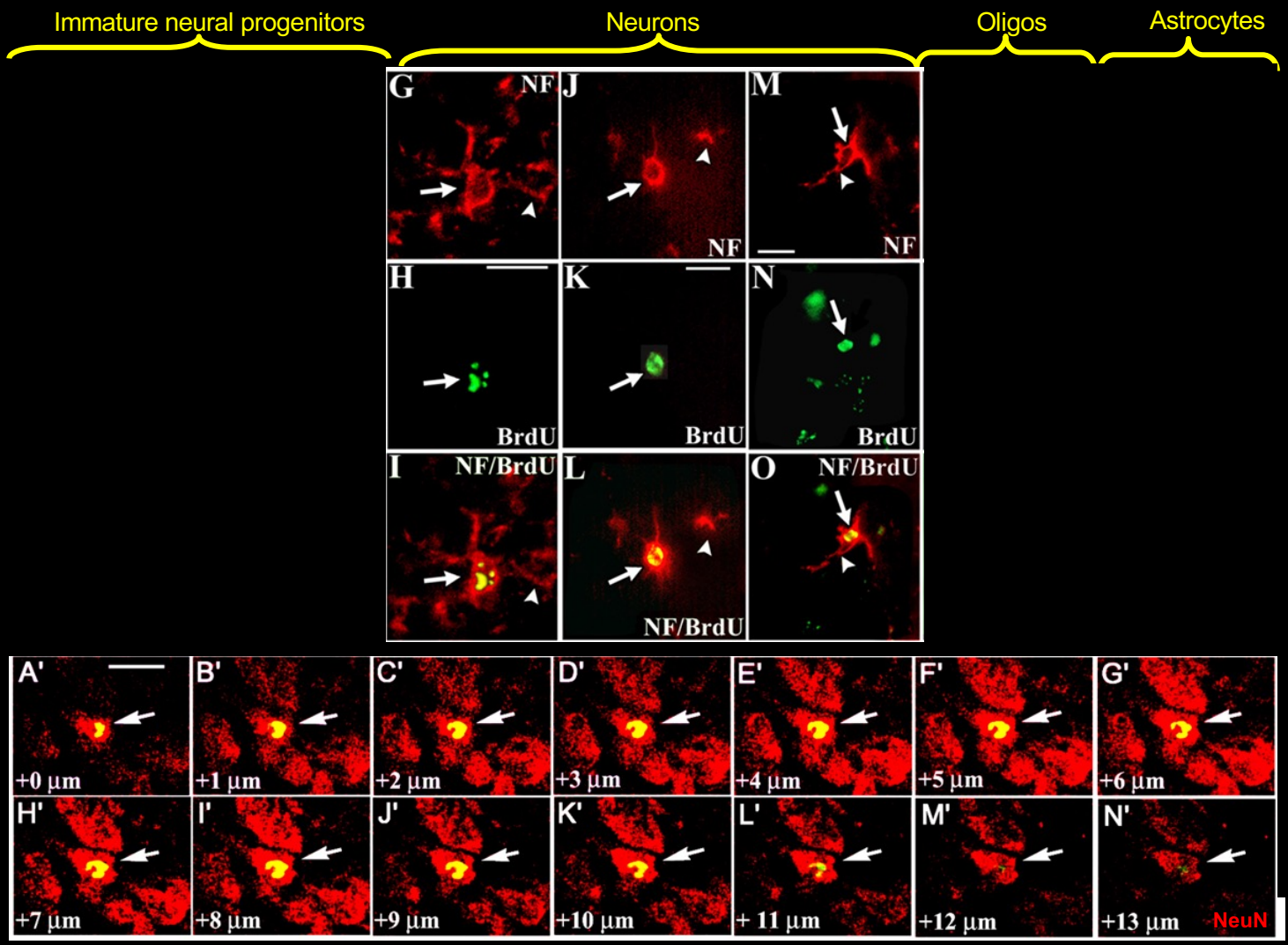
Endogenous NSCs in rodent pup subjected to RVM labeled *in situ* with either BrdU or retrovirally-mediated LacZ to trace their fate



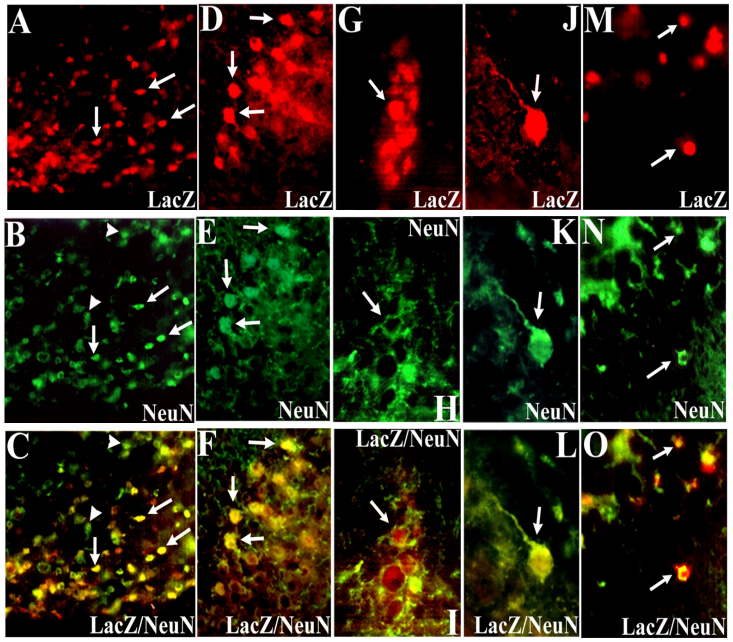


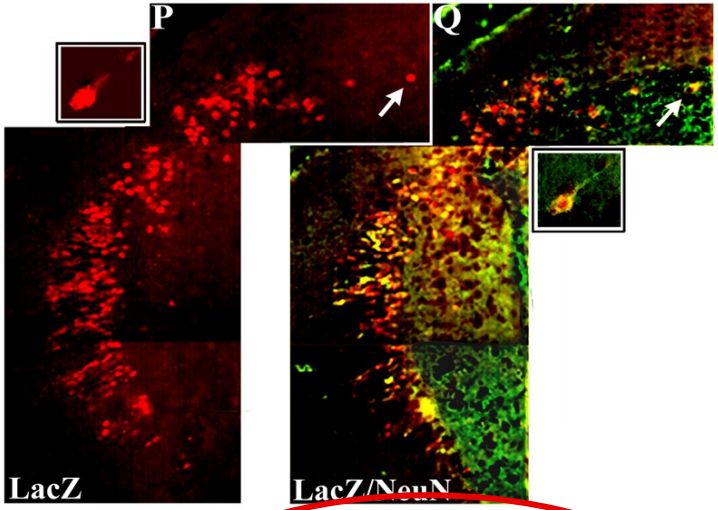
Kook In Park

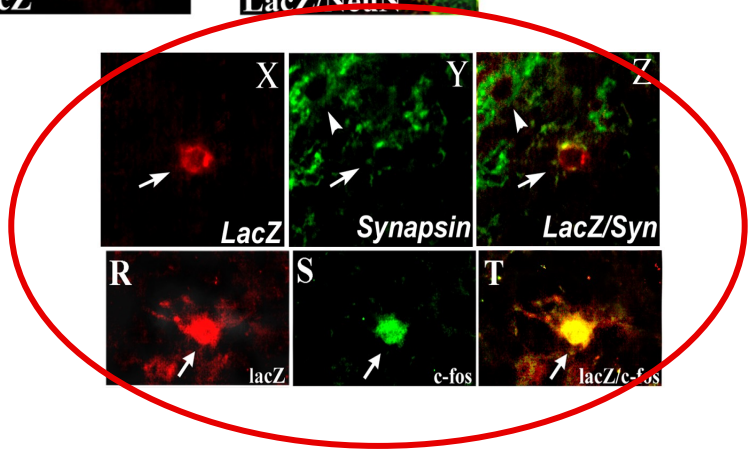



Immature neural progenitors
Neurons
Oligos
Astrocytes
G
NF
J
NF
M
NF
H
BrdU
K
BrdU
N
BrdU
I
NF/BrdU
L
NF/BrdU
O
NF/BrdU
A'
+0 µm
B'
+1 µm
C'
+2 µm
D'
+3 µm
E'
+4 µm
F'
+5 µm
G'
+6 µm
H'
+7 µm
I'
+8 µm
J'
+9 µm
K'
+10 µm
L'
+ 11 µm
M'
+12 µm
N'
+13 µm
NeuN

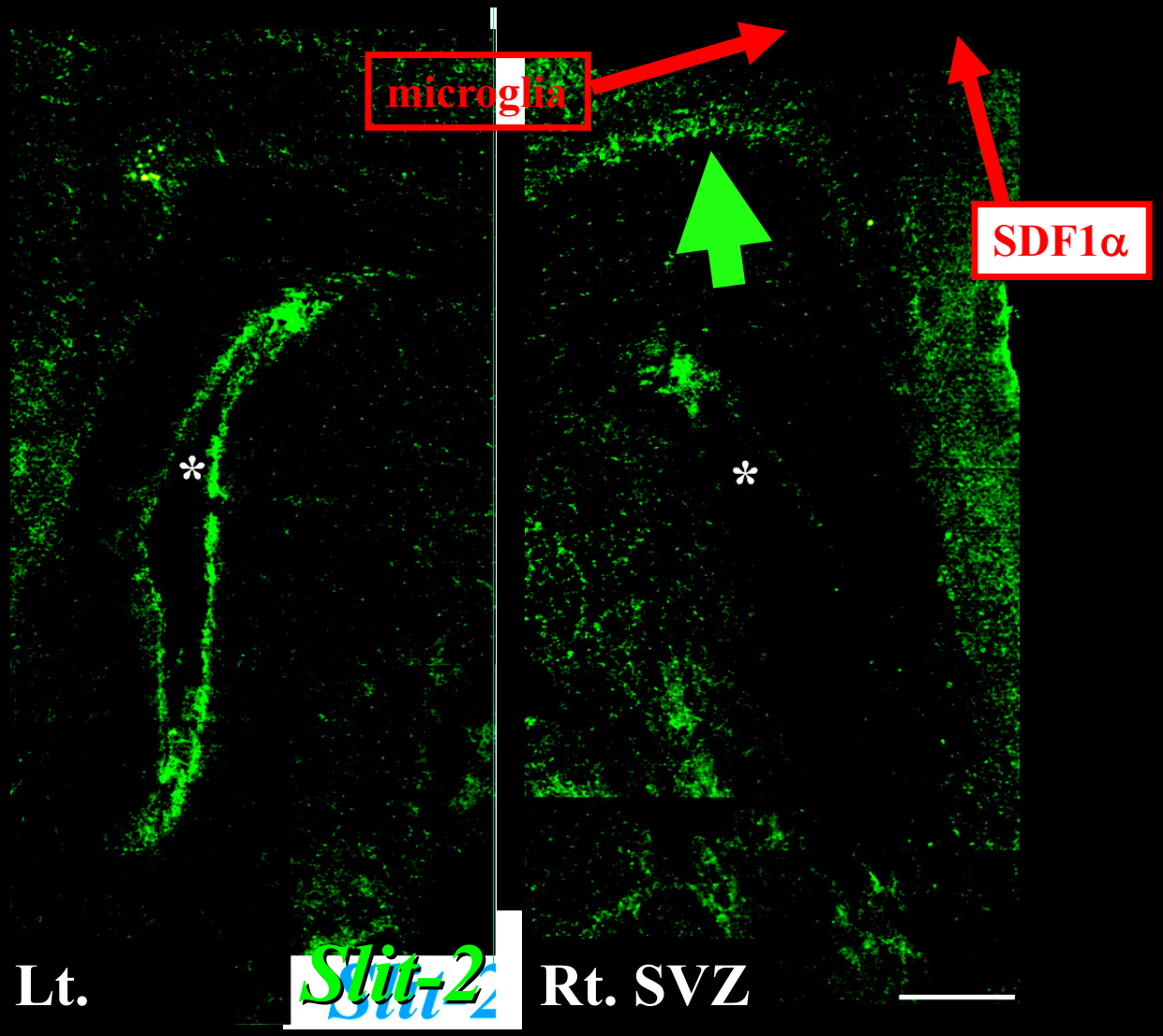
The neurons produced by endogenous NSCs in the penumbra appear to ***be integrated & functional*** based on synapsin decoration & c-fos activation

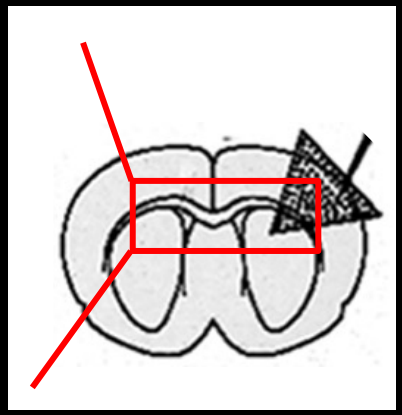






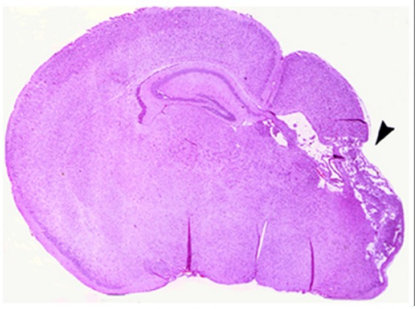
Kook In Park

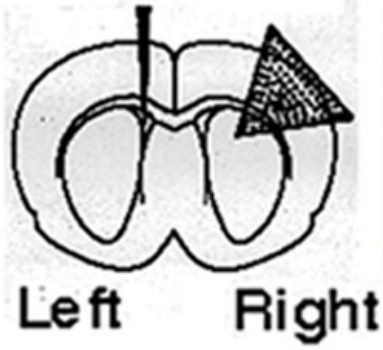

microglia
SDF1α
Slit-2
Lt. SVZ
Rt. SVZ

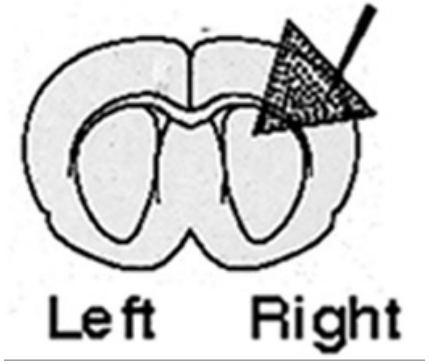


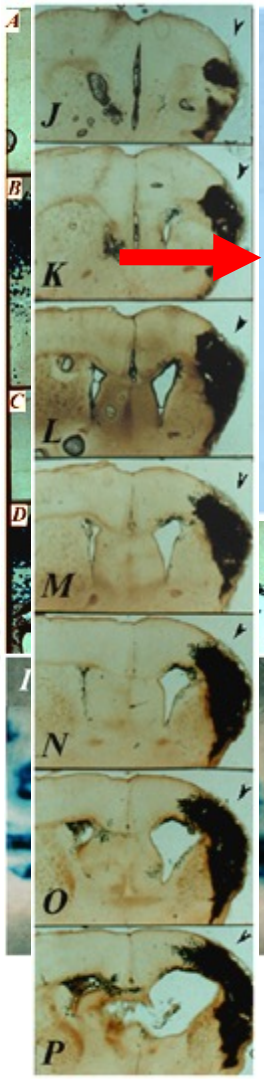
- Intrinsic programs exist in the developing mammalian brain that "attempt" to restore homeostasis.
  - *One of the "teleological" roles of the stem cell*
  - *Perhaps sufficient for some "mild" HII, but "overwhelmed" under severe, even moderate, conditions of injury*
  - *?Augment*

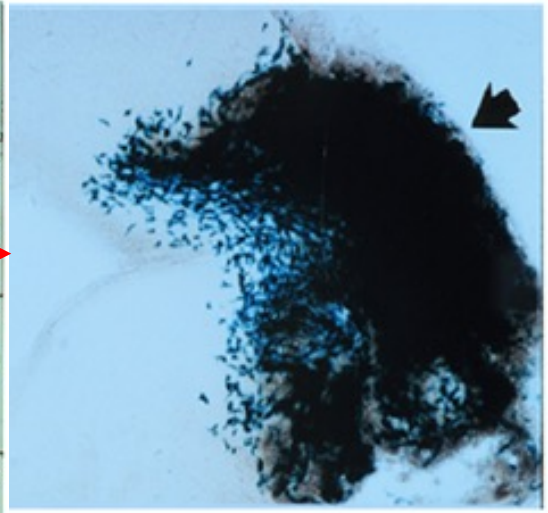
    - Perinatal HII = ideal situation to exploit biology of neural stem cell (NSC) (a component of inherent developmental "programs") in a developing organ with a developmental insult

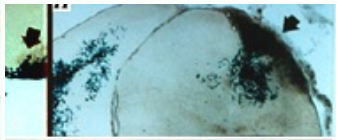


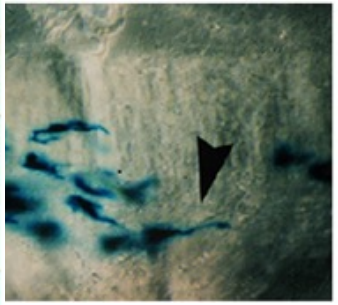












Experimental Neurology

Experimental Neurology 199 (2006) 156–178

Acute injury directs the migration, proliferation, and differentiation of solid organ stem cells: Evidence from the effect of hypoxia–ischemia in the CNS on clonal "reporter" neural stem cells

Kook In Park [a,b], Michael A. Hack [b], Jitka Ourednik [b,c], Booma Yandava [b], Jonathan D. Flax [b], Philip E. Stieg [d], Stephen Gullans [b], Francis E. Jensen [b], Richard L. Sidman [b], Vaclav Ourednik [b,c], Evan Y. Snyder [b,e,*]

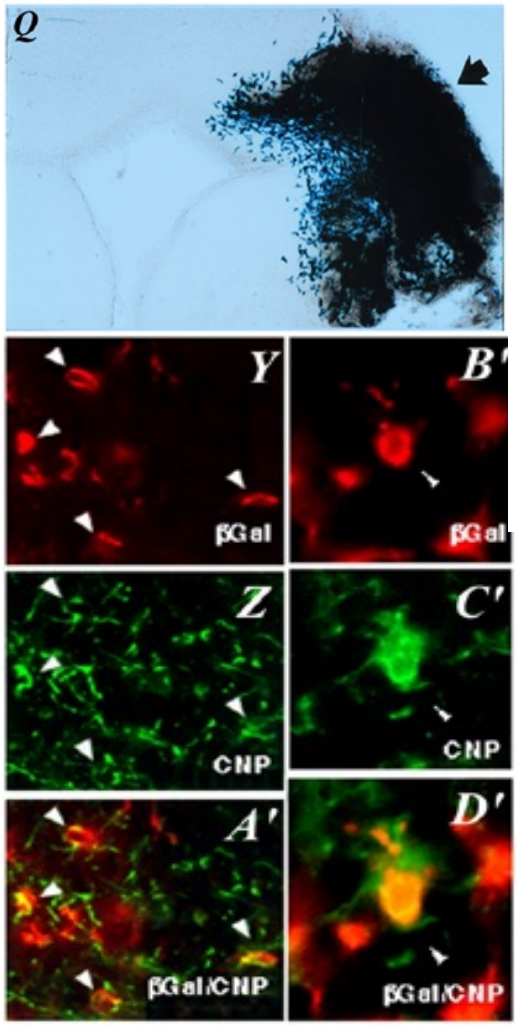
The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue

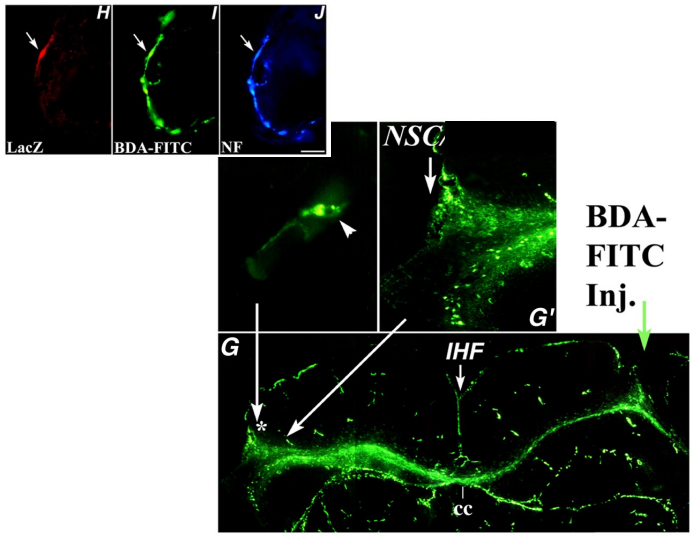
Kook In Park[1,2], Yang D. Teng[2,3], and Evan Y. Snyder[2]*

Published online 15 October 2002; doi:10.1038/nbt751

nature biotechnology

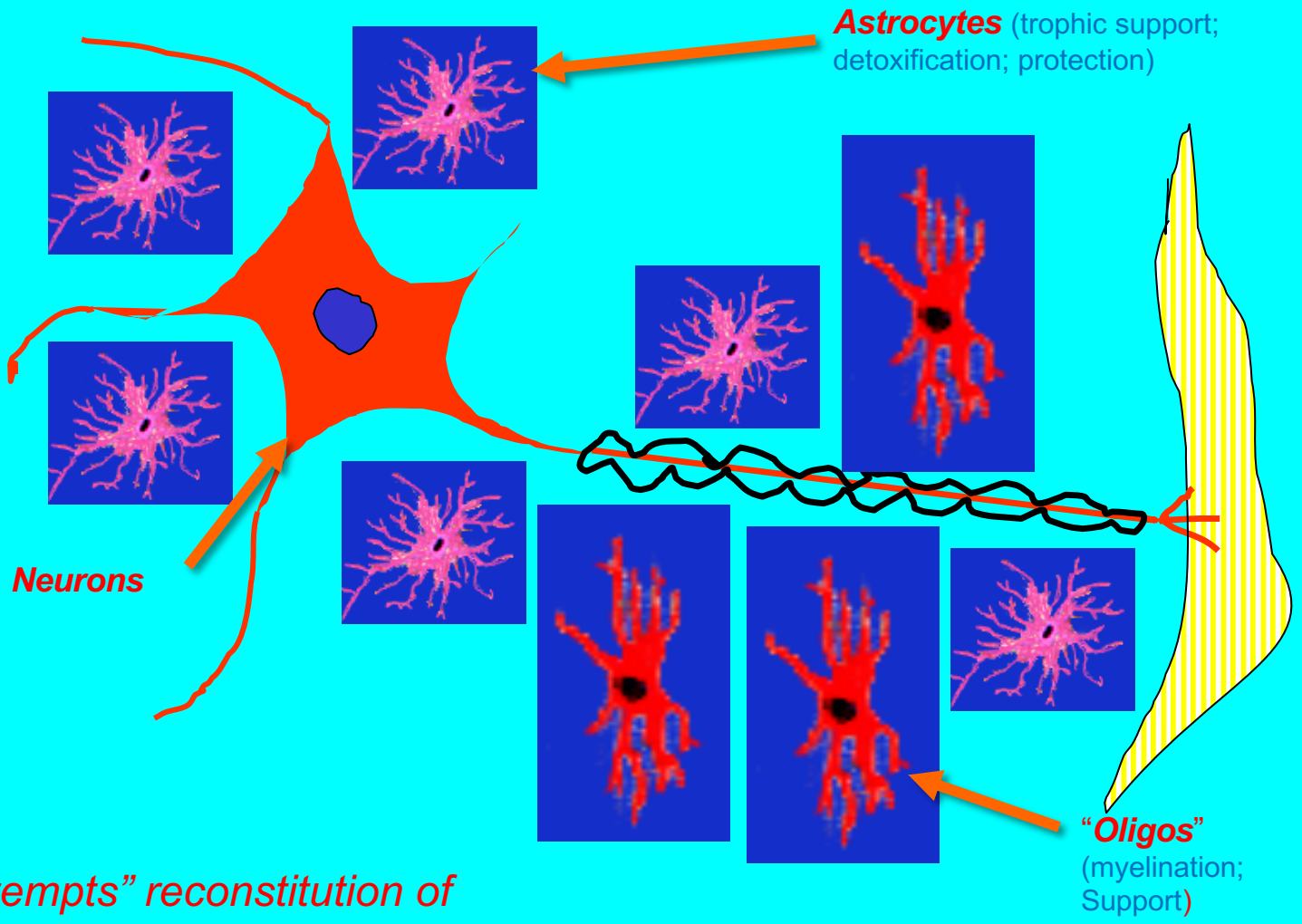
Kook In Park



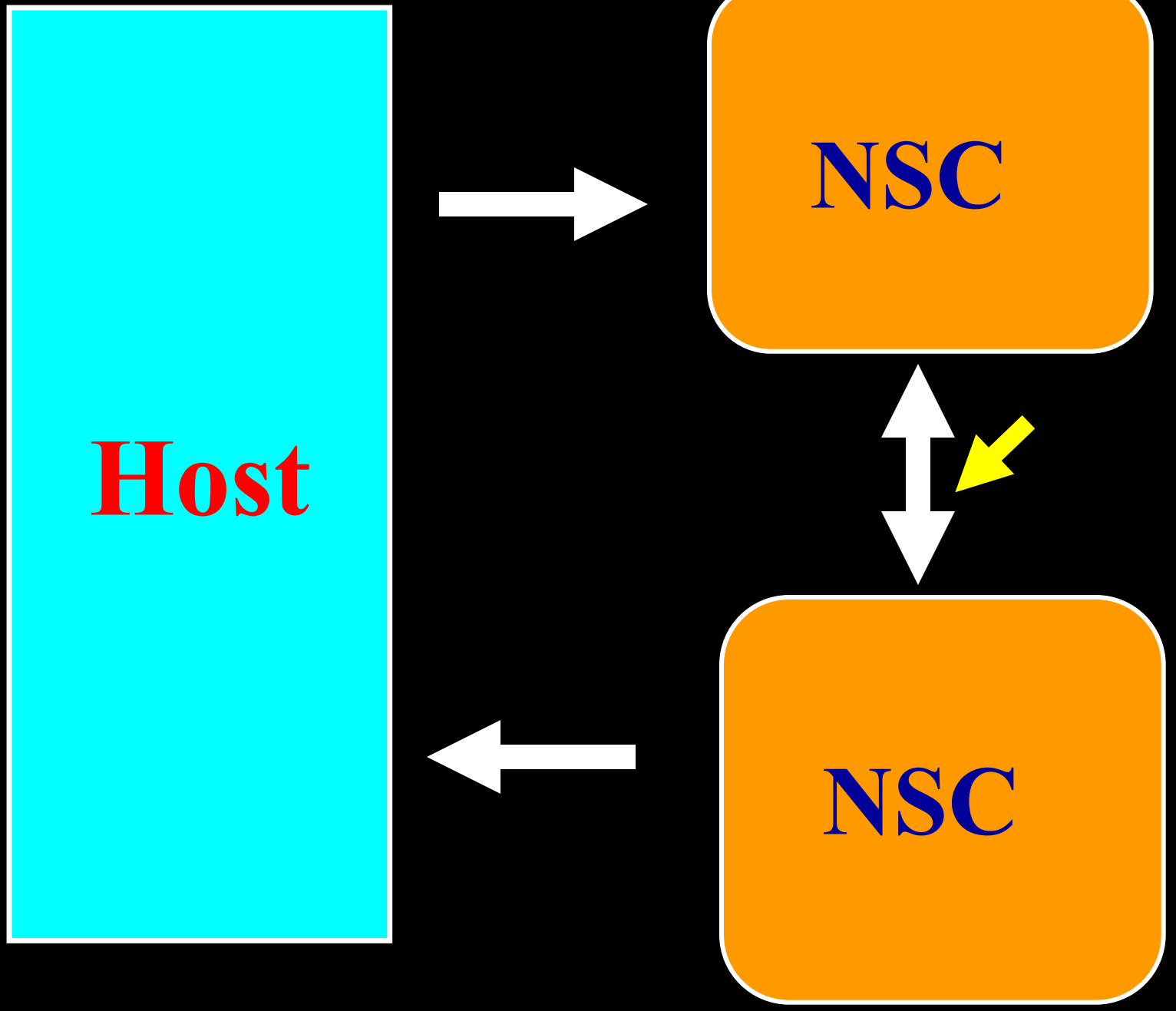


	Intact Left Hemisphere		Hypoxic-Ischemic Right Hemisphere	
Neurons	NeuN	0 %	NeuN	4.6 ± 0.2 %
	MAP-2	0 %	MAP-2	3.3 ± 0.5 %
	NF	0 %	NF	1.3 ± 0.4 %
(oligos)	CNP'ase	0.8 ± 0.2 %	CNP'ase	3.6 ± 0.4 %
(astros)	GFAP	14.7 ± 2.7 %	GFAP	22.5 ± 2.9 %
(NPs)	Nestin	6.0 ± 0.4 %	Nestin	17.2 ± 1.8 %

Park et al, *Nat Biotech*, 2002; *Exp Neurol*, 2006



- *NSC "attempts" reconstitution of all neural cell types of a region in proper ratio & arrangement*
- Likely *all* cells in system needed to restore function and/or redress disease


Host
NSC
NSC

NSC

nature biotechnology

RESEARCH ARTICLE

Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons

Jitka Ourednik[1,2,5*†], Václav Ourednik[1,2,5†], William P. Lynch[3], Melitta Schachner[1,4‡], and Evan Y. Snyder[2*‡]

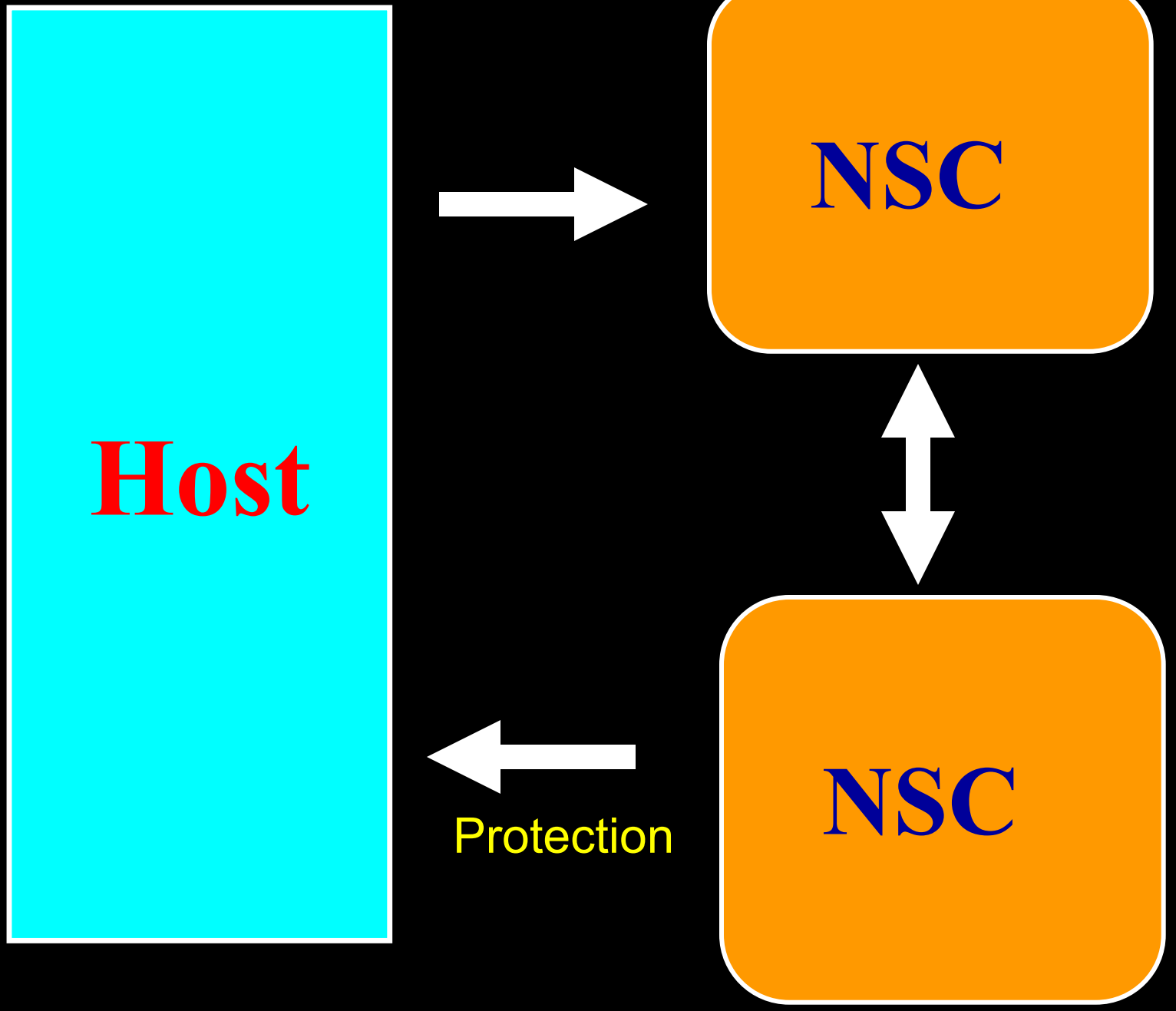
Published online 15 October 2002; doi:10.1038/nbt750

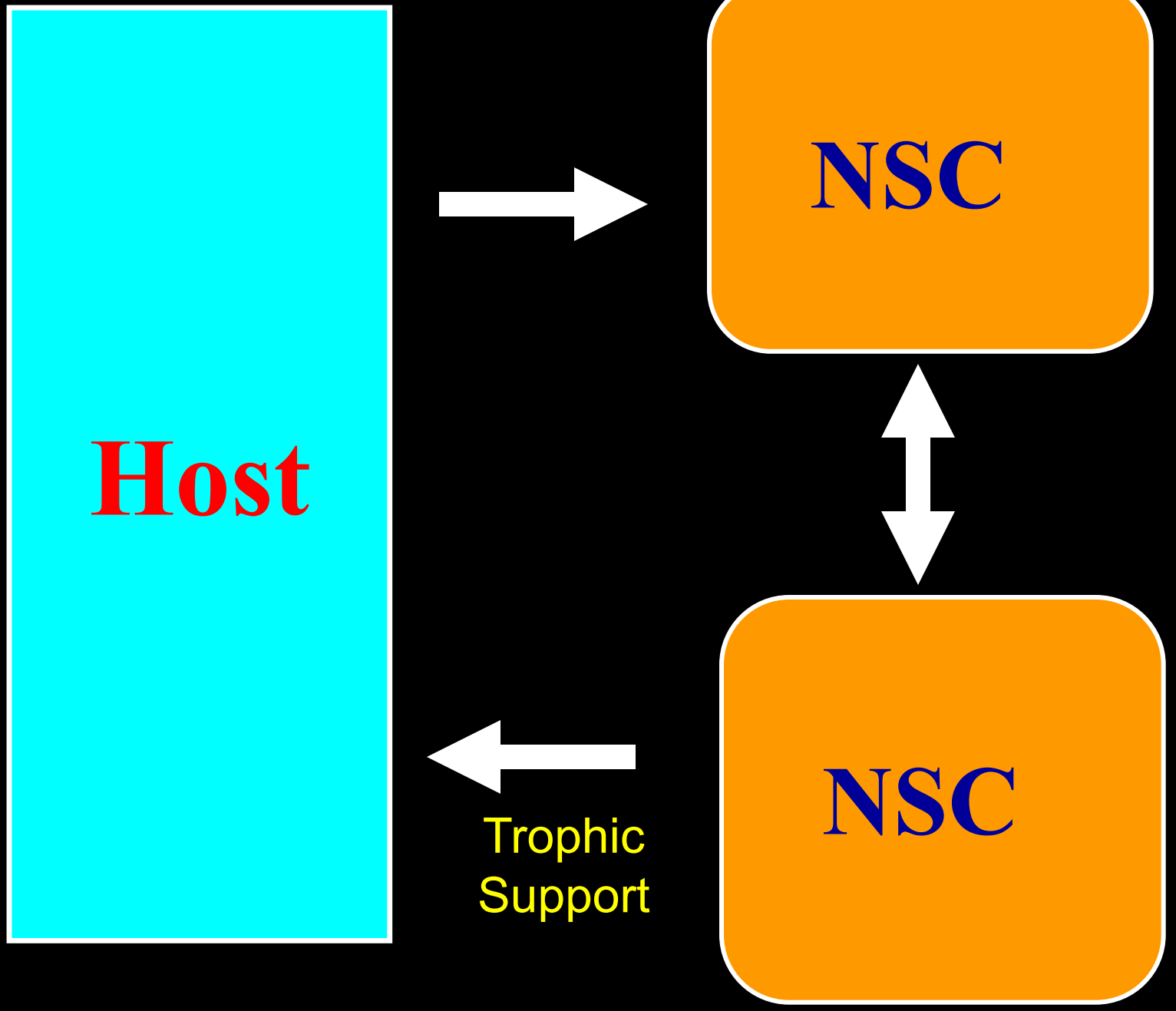
*"Chaperone Effect"**

NSC

Vaclav & Jitka Ourednik

*Ourednik et al *Nat Biotech*, 2002; Park et al, *Nat Biotech*, 2002


Host
NSC
NSC
Protection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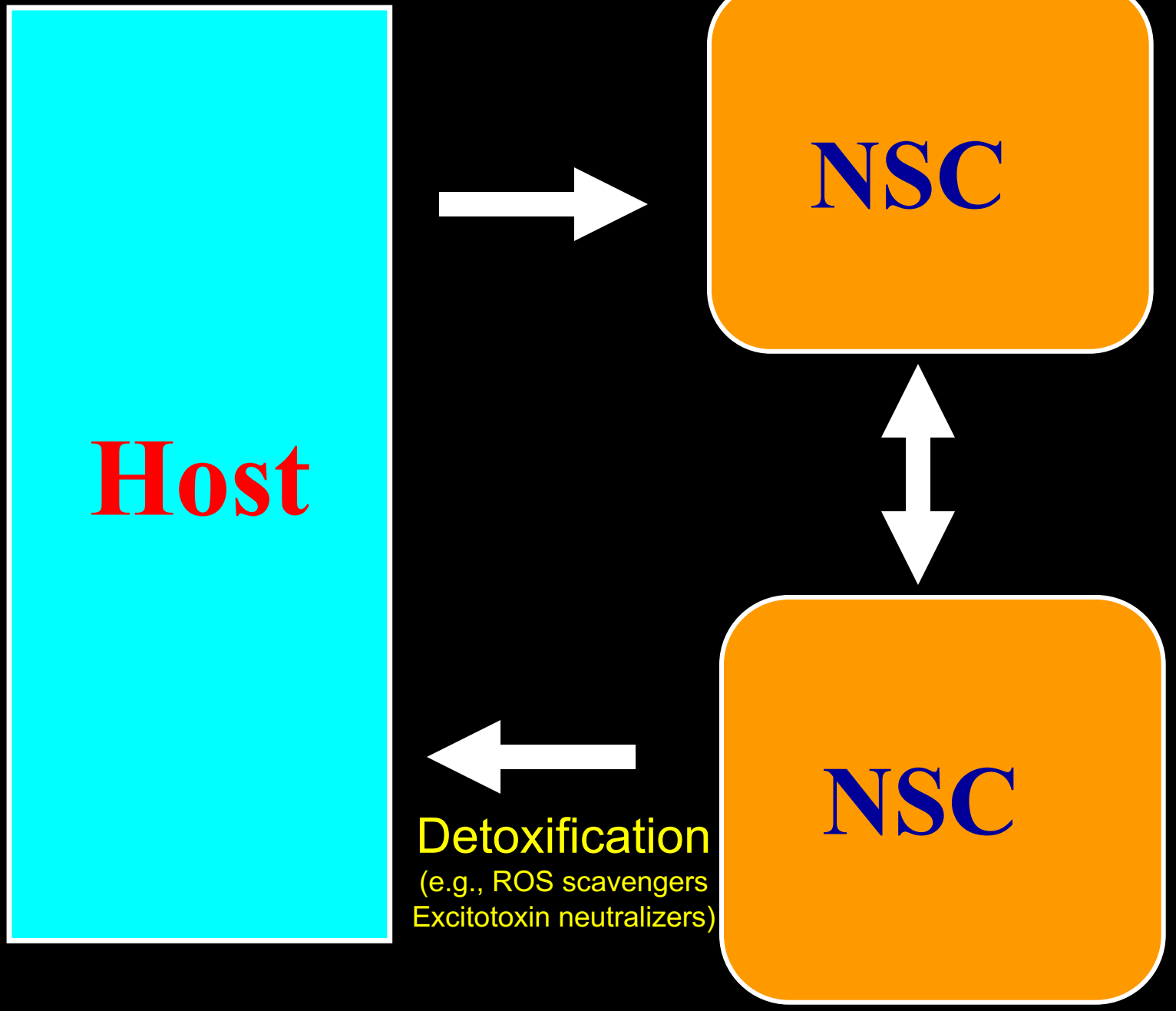
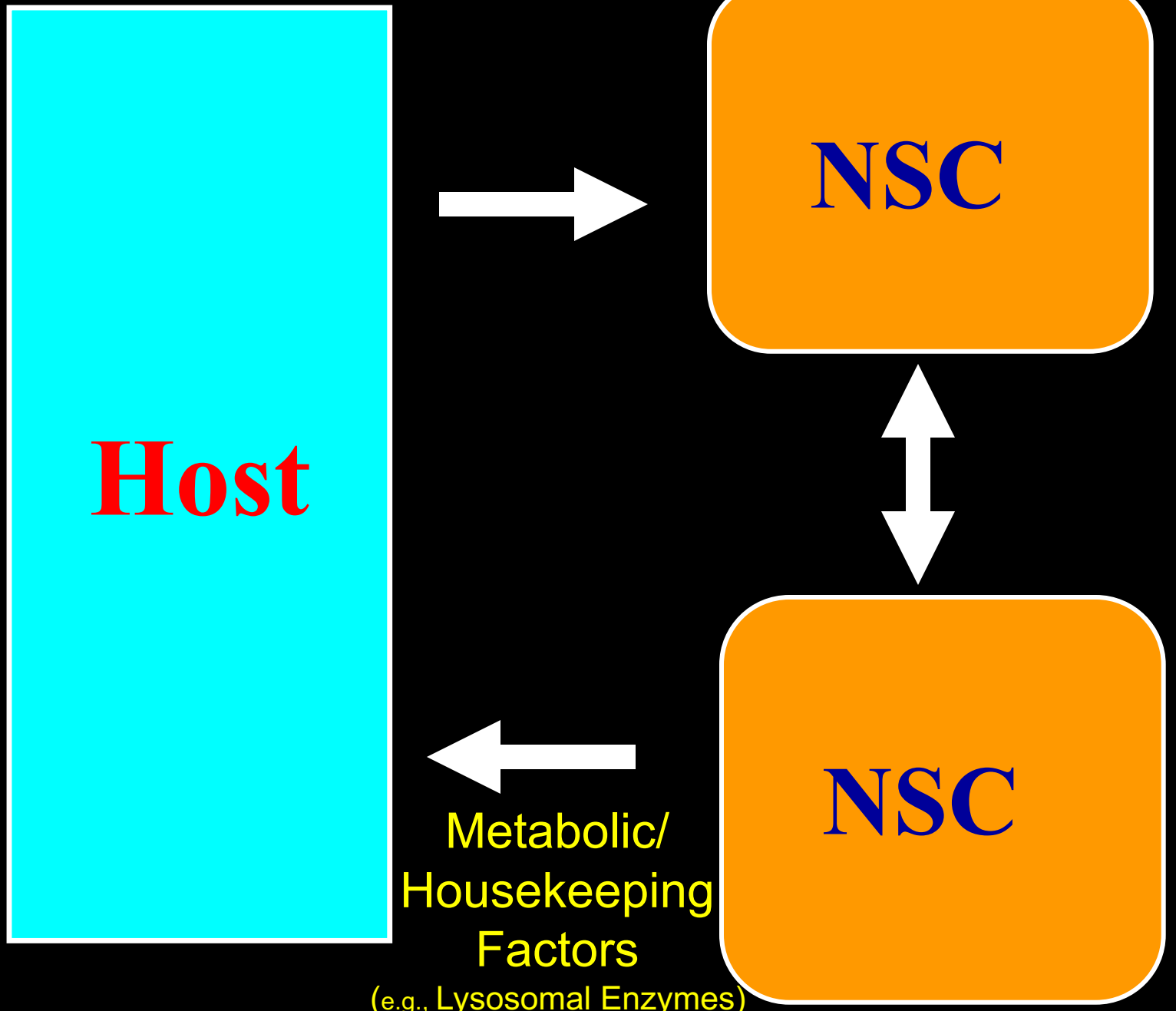
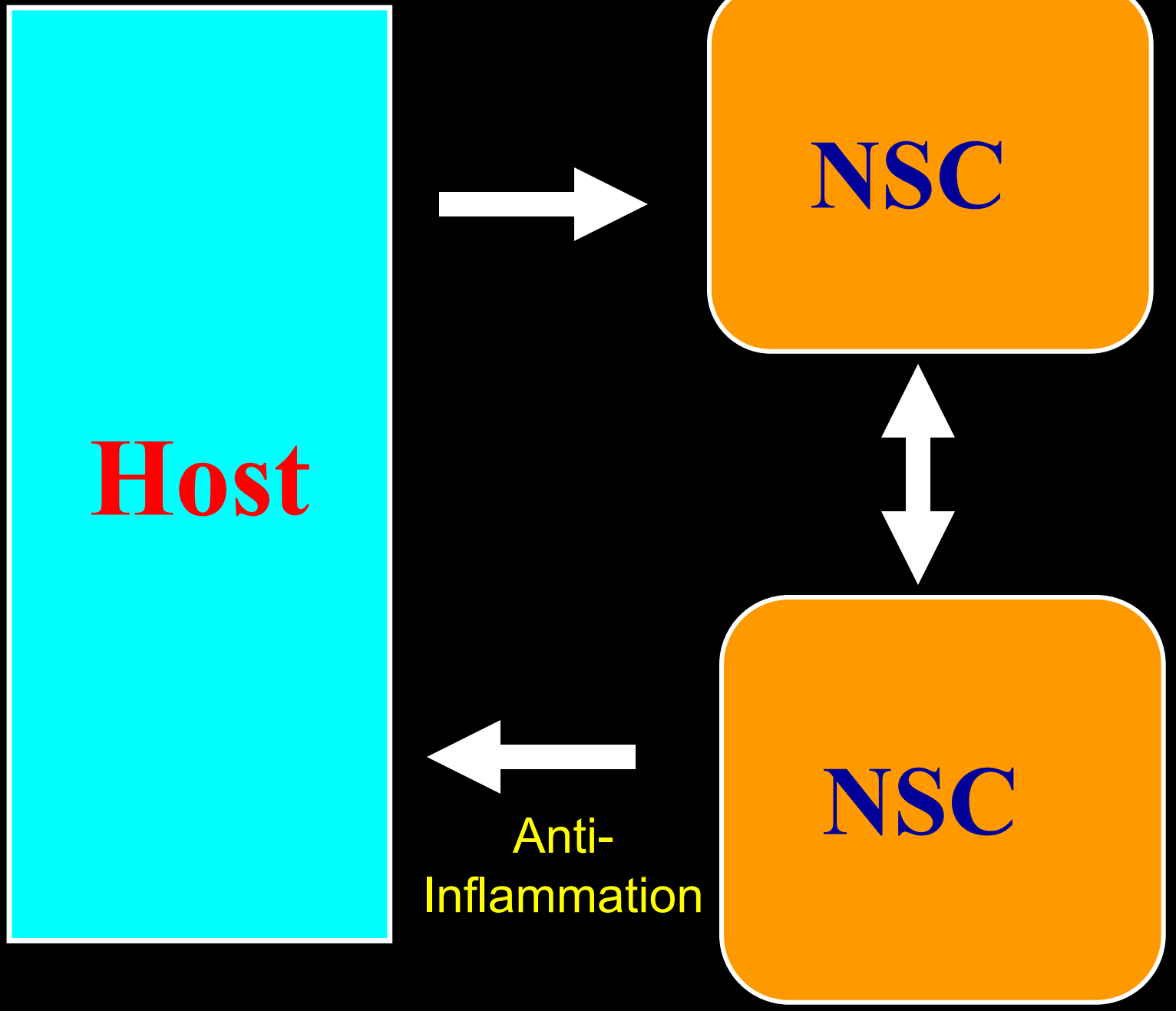
NSC
Trophic
Support


Host
NSC
NSC
Detoxification
(e.g., ROS scavengers
Excitotoxin neutralizers)


Host
NSC
NSC
Metabolic/
Housekeeping
Factors
(e.g., Lysosomal Enzymes)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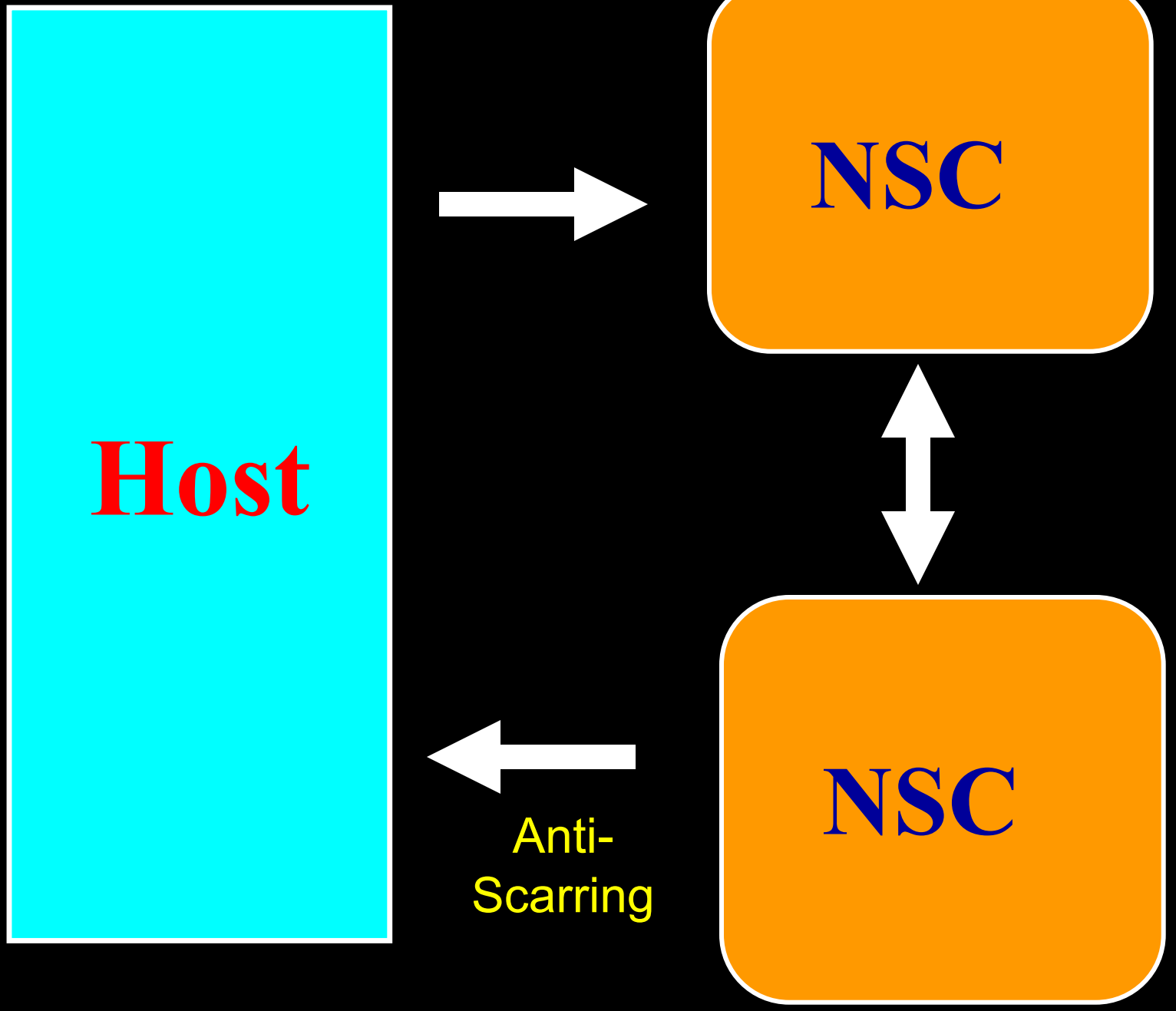
NSC
Anti-
Inflammation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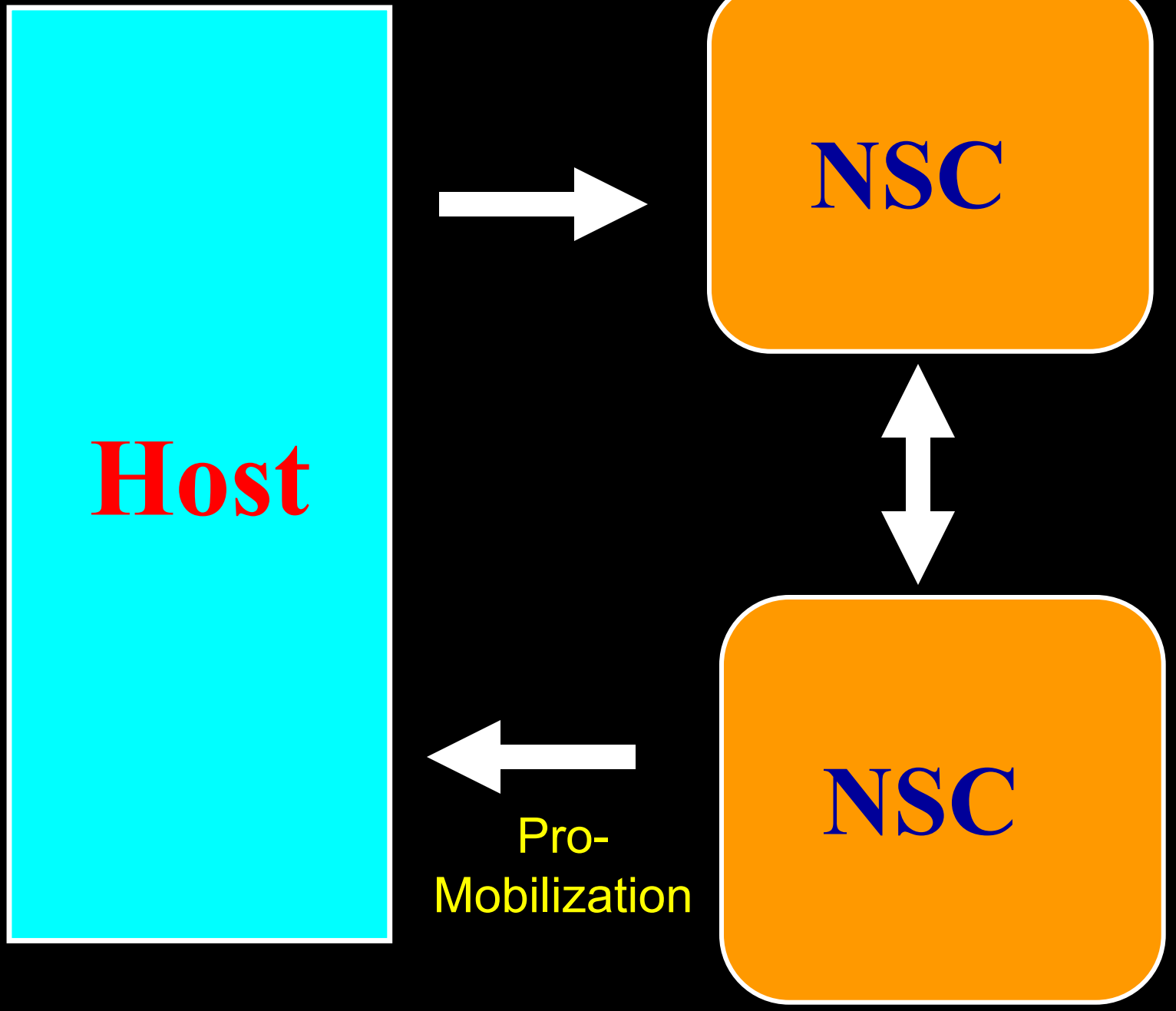
NSC
Anti-
Scarring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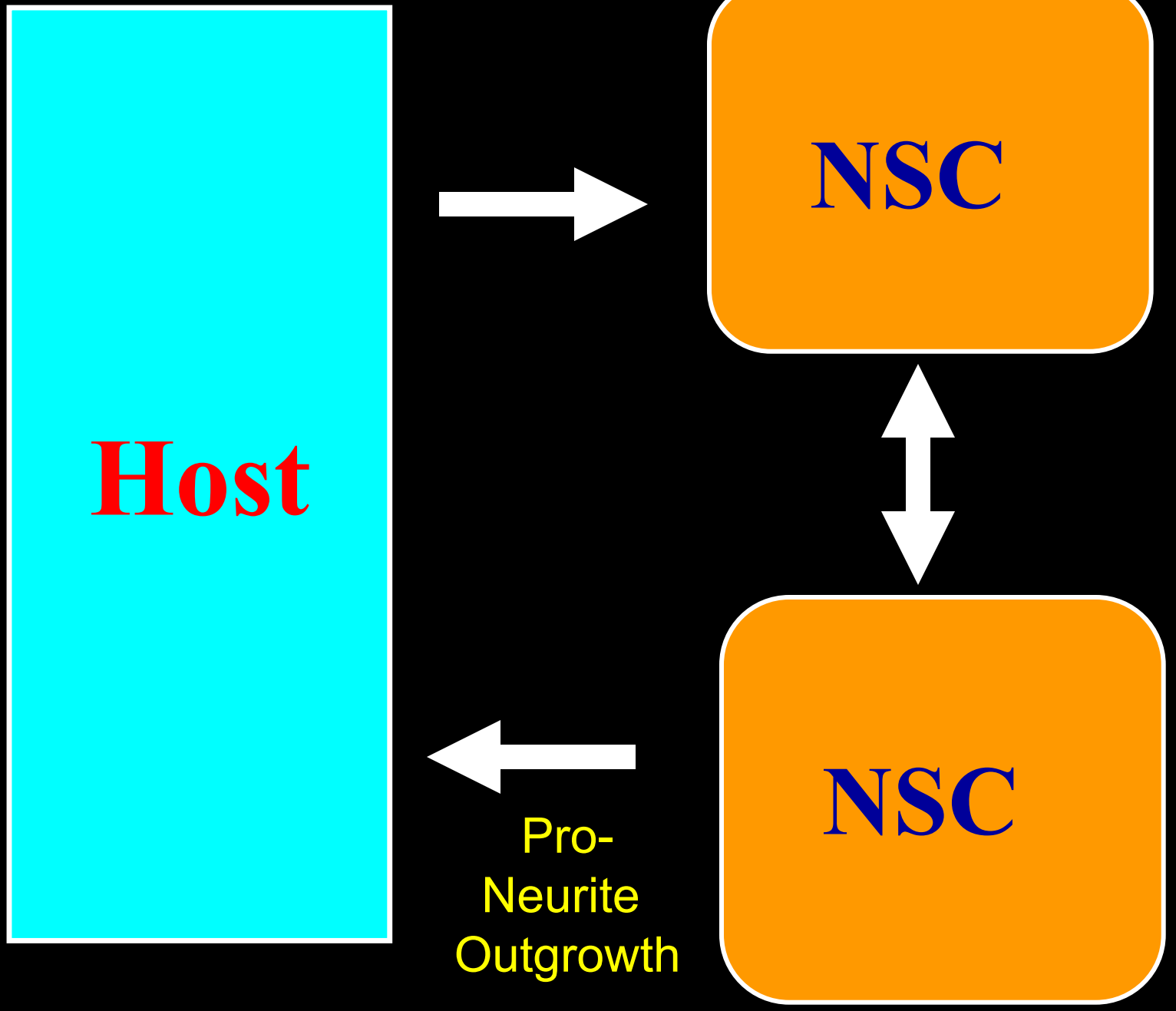
NSC
NSC
Pro-
Mobilization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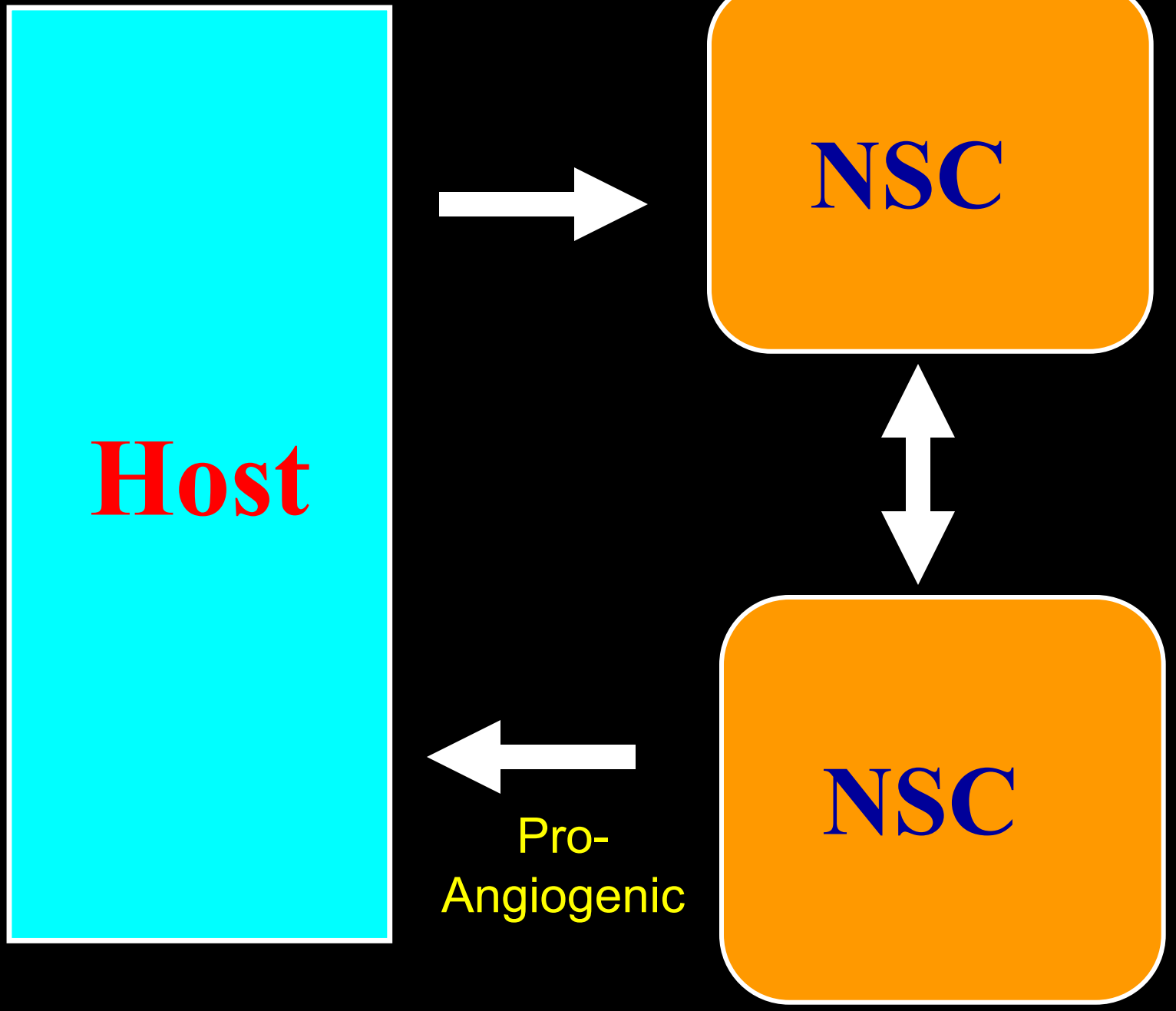
NSC
NSC
Pro-
Neurite
Outgrowth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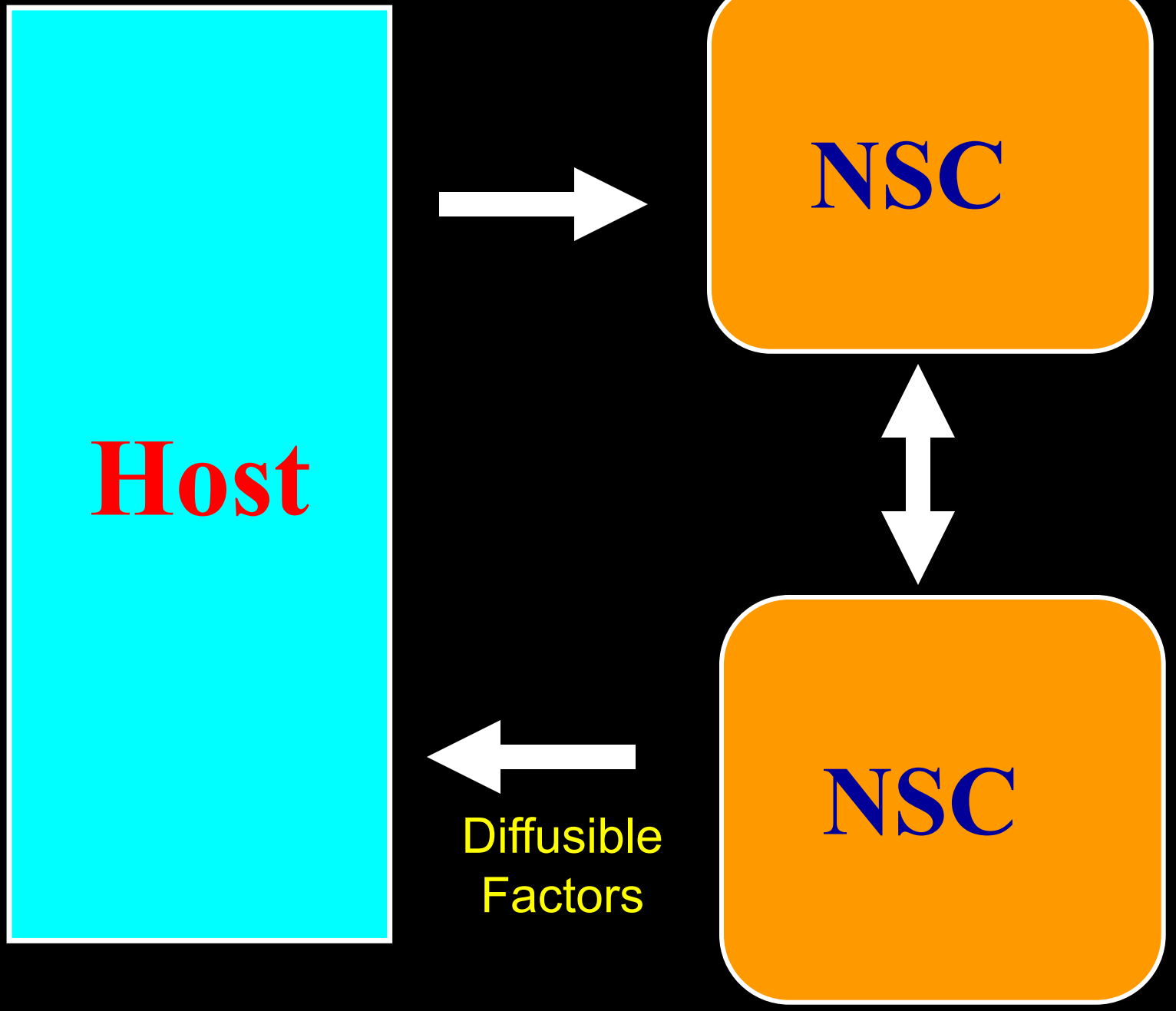
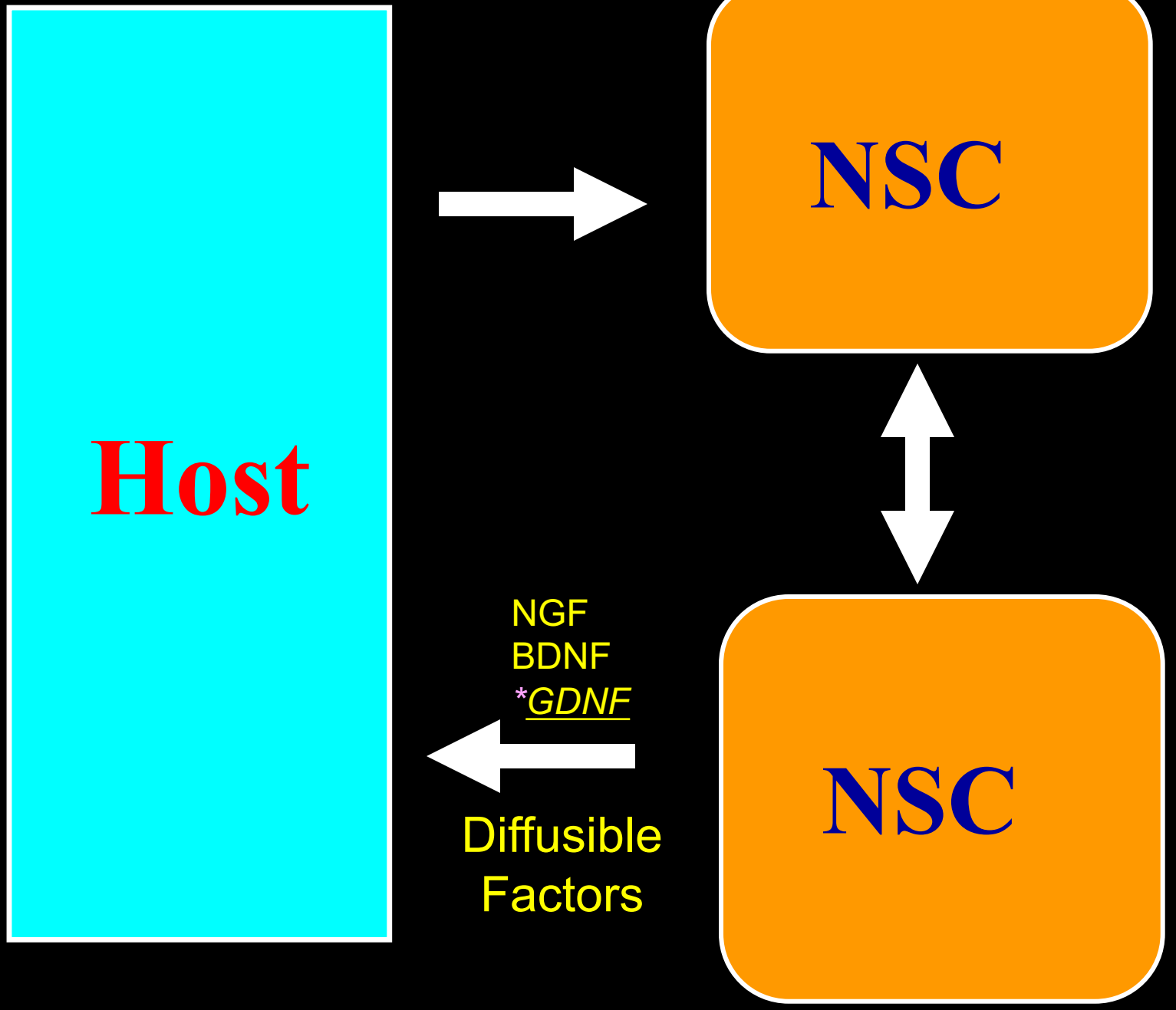
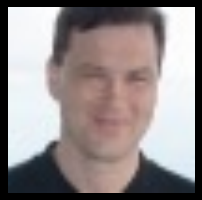
NSC
NSC
Pro-
Angiogenic

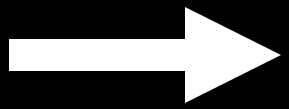

Host
NSC
NSC
Diffusible
Factors

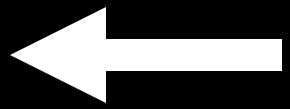

Host
NSC
NSC
NGF
BDNF
*GDNF
Diffusible
Factors



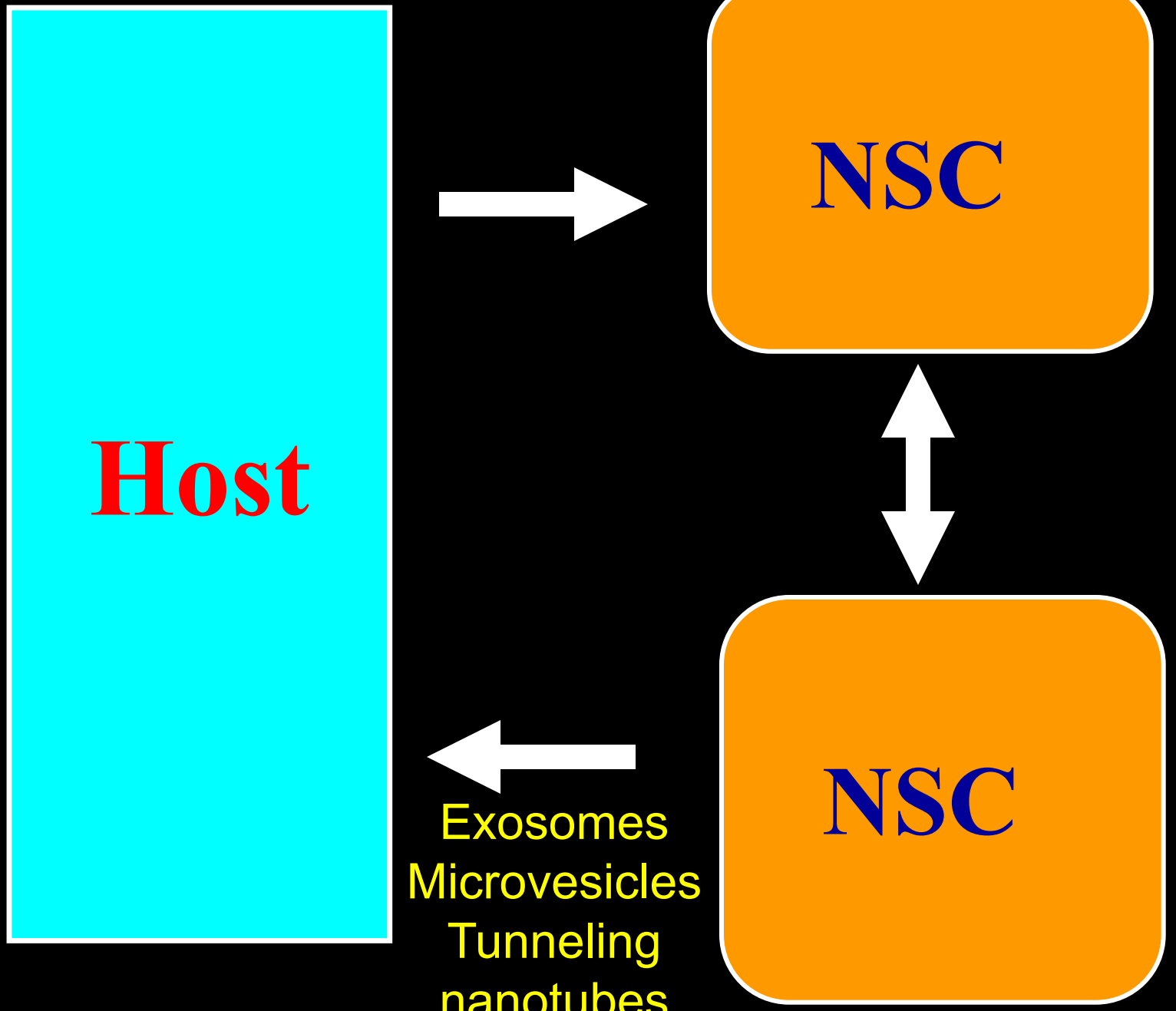
Eric Herlenius

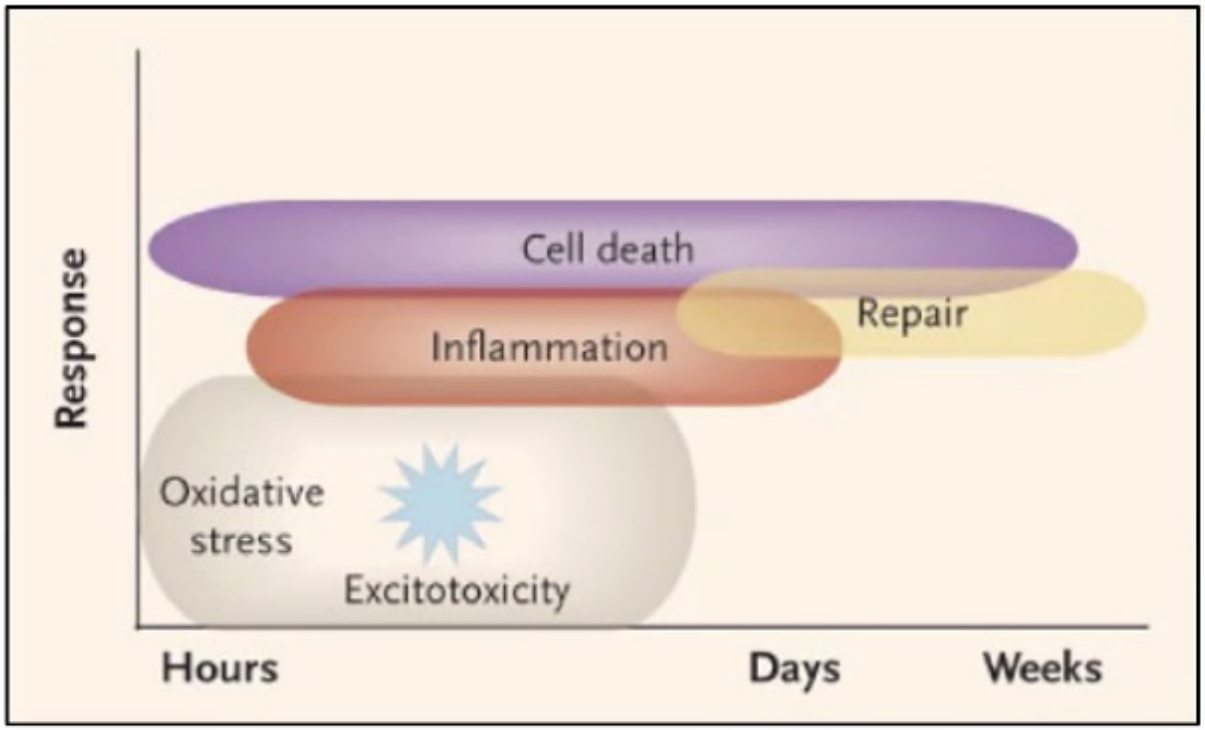
Jaderstad et al, *PNAS* (2010)

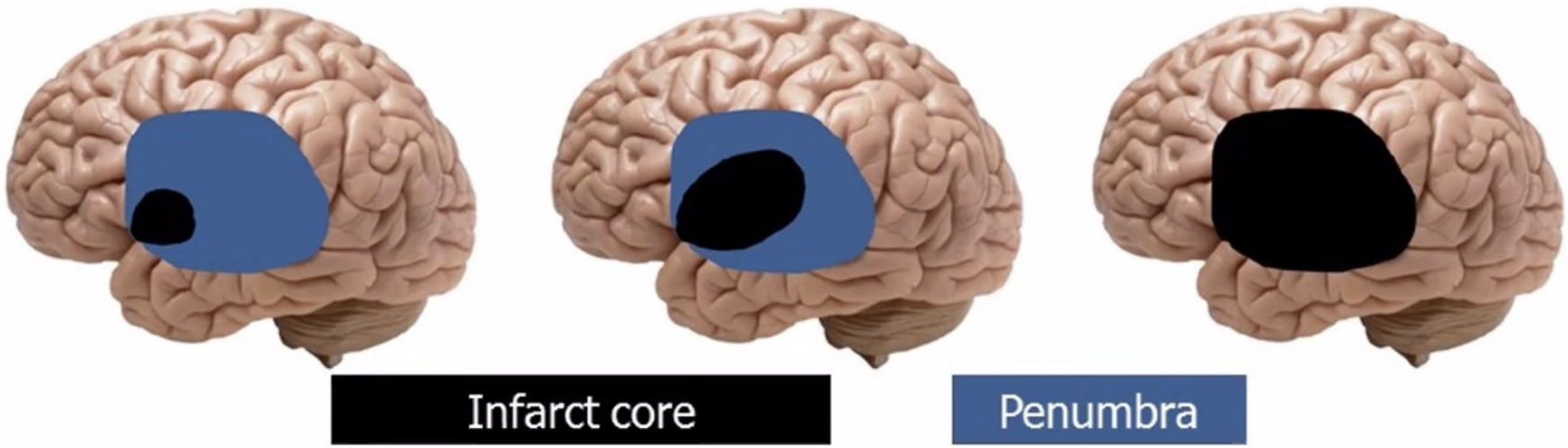




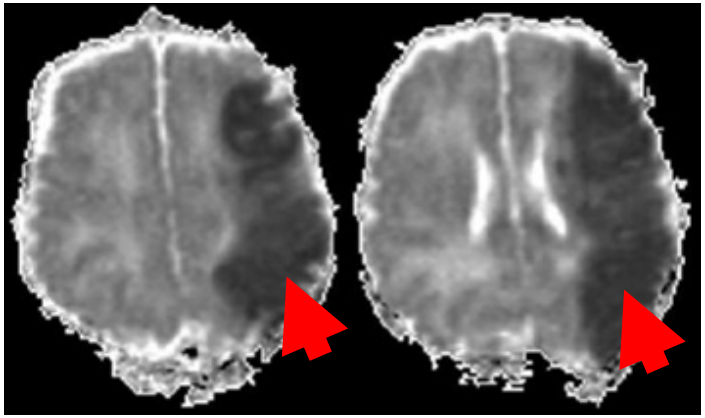
Cell-Cell
Contact
(gap junctions)

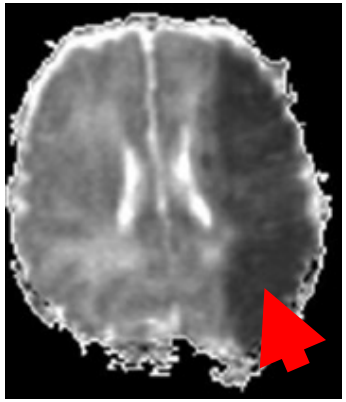

Host
NSC
NSC
Exosomes
Microvesicles
Tunneling
nanotubes





Donna Ferriero, *NEJM*





ORIGINAL ARTICLE

Automated core–penumbra quantification in neonatal ischemic brain injury

Nirmalya Ghosh[1], Xiangpeng Yuan[1], Christine I Turenius[1], Beatriz Tone[1], Kamalakar Ambadipudi[2], Evan Y Snyder[3], Andre Obenaus[1,4] and Stephen Ashwal[1]

Journal of Cerebral Blood Flow & Metabolism (2012), 1–10

For us, *mechanistic "breakthrough"* was not solely recognizing that these lesions were *not homogeneous*, but that we could – in real-time, in *living* animals (& patients) – *subdivide* lesion into regions – especially *salvageable penumbra* & *irretrievable necrotic core* – & see what NSCs were doing


Andy Obenaus

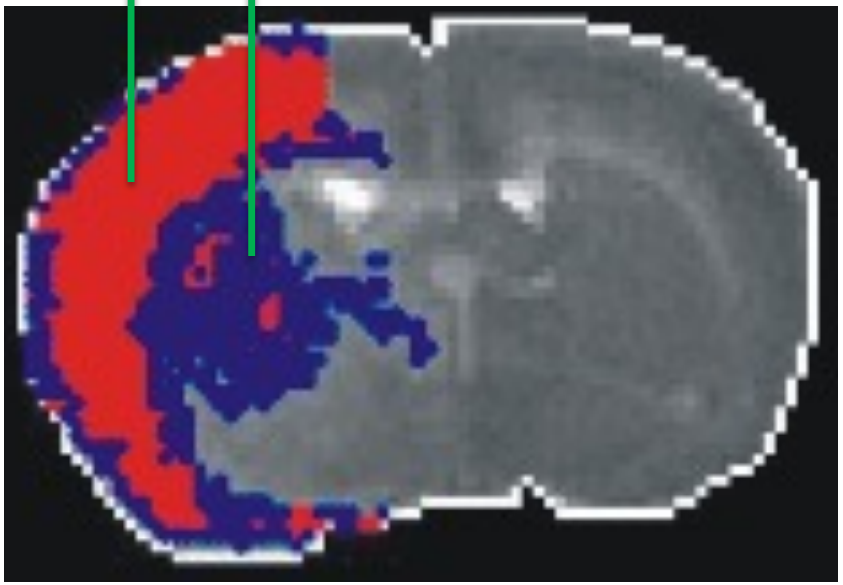

Steve Ashwal


Nirmalya Ghosh

Magnetic resonance imaging (MRI) of an acutely ischemic brain

Core (Neurons already dead; unsalvageable; molecularly "silent")

Penumbra (Neurons "hurt", but not dead; might be rescued by neuroprotective stem cells; molecularly "active)"



via

Hierarchical Region Splitting (HRS)

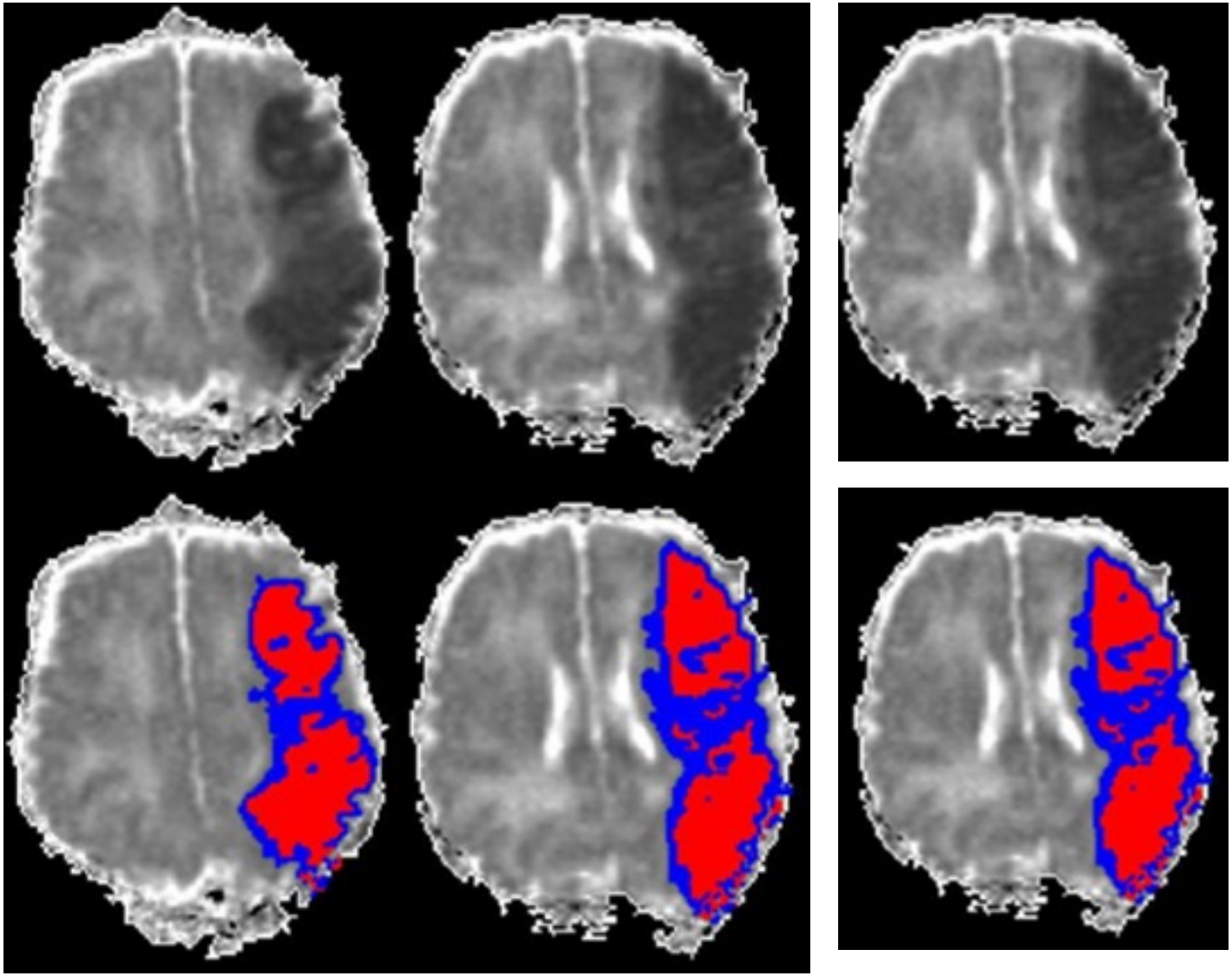
= T2WI+DWI

(average diffusion coefficient [ADC])

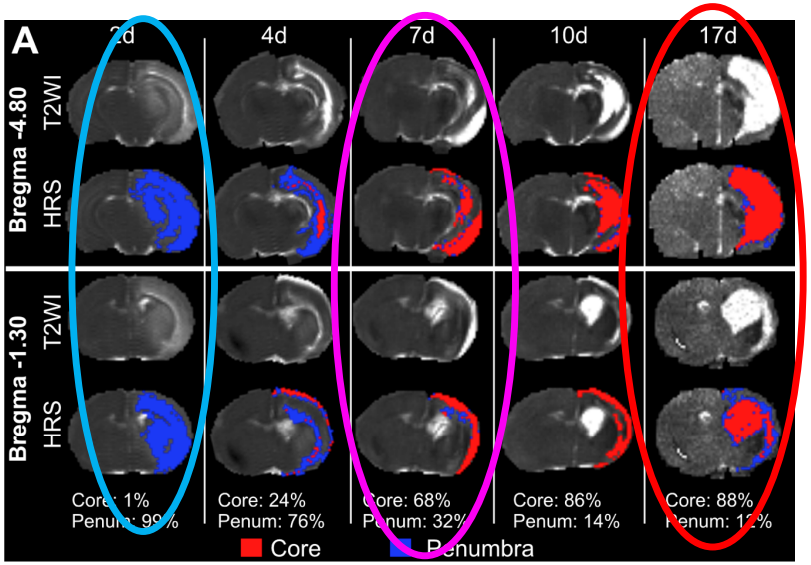
Andy Obenaus

Steve Ashwal

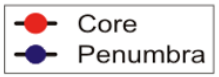
Nirmalya Ghosh

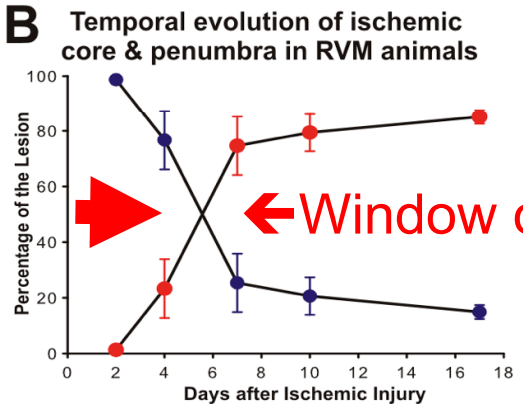


Natural history of Perinatal Hypoxic-Ischemic Injury



- *Initially:* salvageable **Penumbra** >> necrotic **Core**
- *D 4 ➔7:* **Penumbra** dying ➔ necrotic **Core**
- *By D 17:* **Penumbra** largely ➔ unreclaimable **Core**



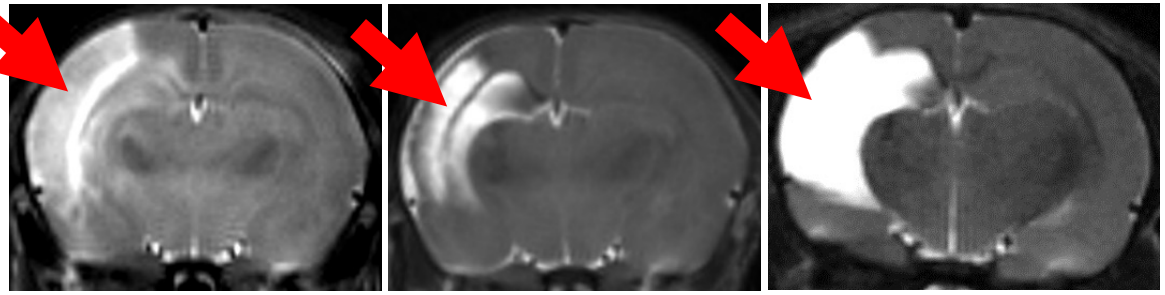


➔Window of opportunity for rescue?

Ghosh et al, *J Cerebral Blood Flow Metab*, 2012

HI lesion ***progression*** when hypothermia is followed by administration of only vehicle or conditioned medium* controls

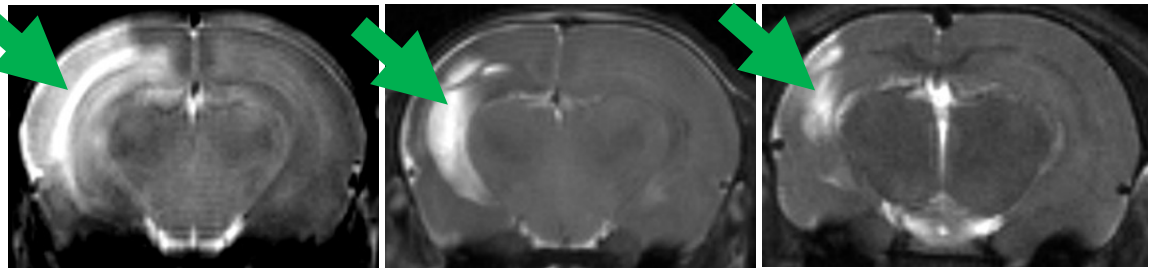
A



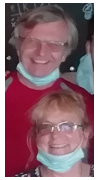
PLD2 PLD5 PLD32

HI lesion ***reduction*** when hypothermia is followed by hNSC administration

B



PLD2 PTrD3 PLD32/PTrD30



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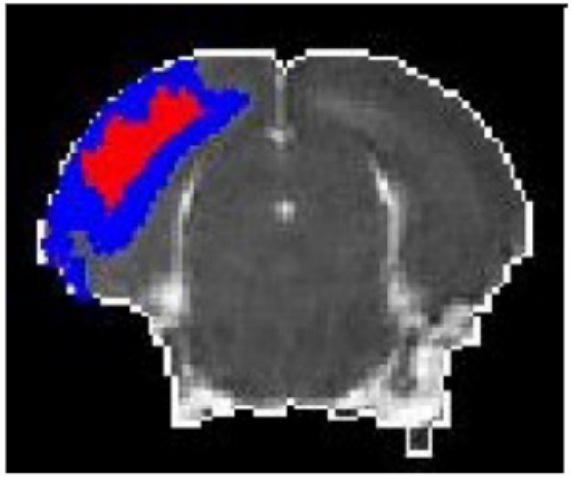
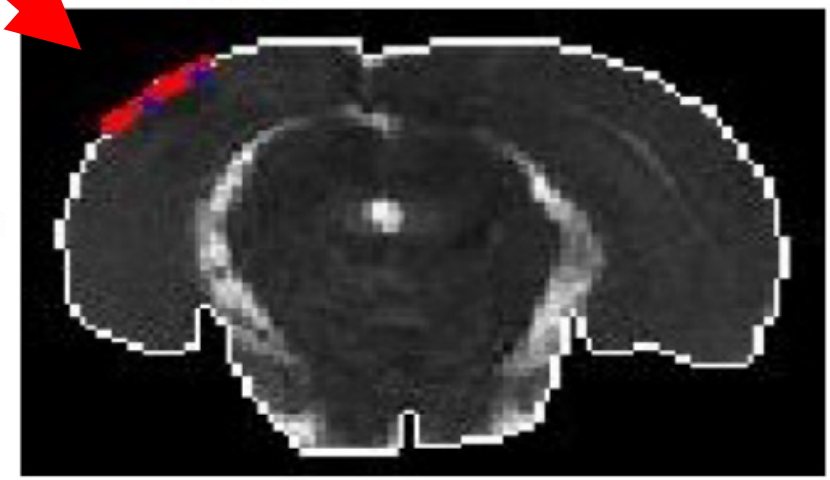
*speaks *against* simply secreted neurotrophic factors or exosomes

Significant neuroprotection conferred by intraventricular hNSC grafts on reversing severity or suppressing progression of severity of the HI lesions in RVM rats

PLD2, moderate lesion, no graft

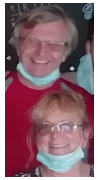


PTD90, graft - lesion reduction



Moderate lesion:
9.01% (from brain vol)
2.13% (core, red)
6.88% (penumbra, blue)

Graft-reduced lesion:
0.29% (from brain vol)
0.20% (core, red)
0.09% (penumbra, blue)



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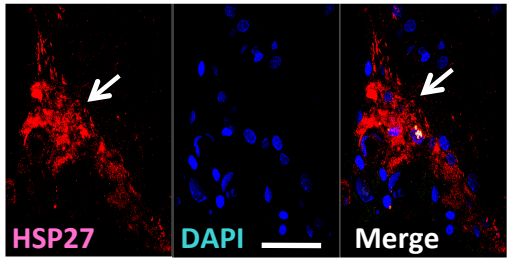
No immunosuppression (hNSCs lack MHC-II)

Heat Shock Protein-27 (HSP27) expression (*"reparative biomarker"*) is positively-related to *Severity* & injury site – expressed in – and *only* in – the ***penumbra***

(to where hNSCs drawn)



Even donor hNSCs came to express **HSP27** once engrafted (though not before)


Cameron Pernia

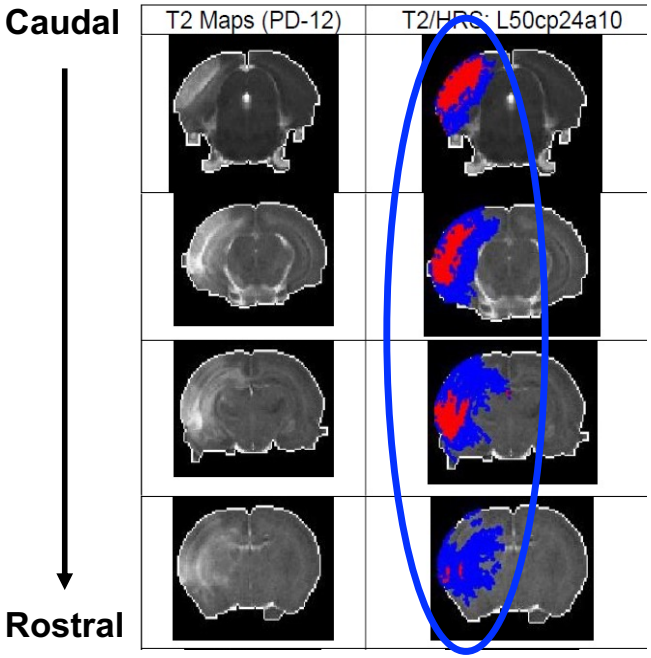

Neal Nathan

Hartman et al, *Cell Reports* (2020)

Resolution of HI lesion when hNSCs administered following HT

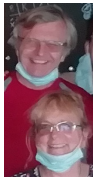
Penumbral tissue (blue) is normalized (size decreases)

(Irretrievable necrotic core (red) remains, albeit somewhat diminished)



PD12

Core: 4.41%, penumbra: 8.70%



Vaclav & Jitka Ourednik

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Report

A Biomarker for Predicting Responsiveness to Stem Cell Therapy Based on Mechanism-of-Action: Evidence from Cerebral Injury

Richard E. Hartman,[1] Neal H. Nathan,[2,3] Nirmalya Ghosh,[4] Cameron D. Pernia,[2,3] Janessa Law,[2,3,5] Ruslan Nuryyev,[2,3] Amy Plaia,[4] Alena Yusof,[4] Beatriz Tone,[4] Melissa Dulcich,[1] Dustin R. Wakeman,[2] Nejmi Dilmac,[2] Walter D. Niles,[2] Richard L. Sidman,[6] Andre Obenaus,[4,7,8] Evan Y. Snyder,[2,3,5,9,*] and Stephen Ashwal[4,*]

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[2]Center for Stem Cells & Regenerative Medicine, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA
[3]Sanford Consortium for Regenerative Medicine, La Jolla, CA 92037, USA
[4]Department of Pediatrics, Loma Linda University, Loma Linda, CA 92350, USA
[5]Department of Pediatrics, University of California, San Diego, La Jolla, CA 92037, USA
[6]Department of Neurology, Harvard Medical School, Boston, MA 02115, USA
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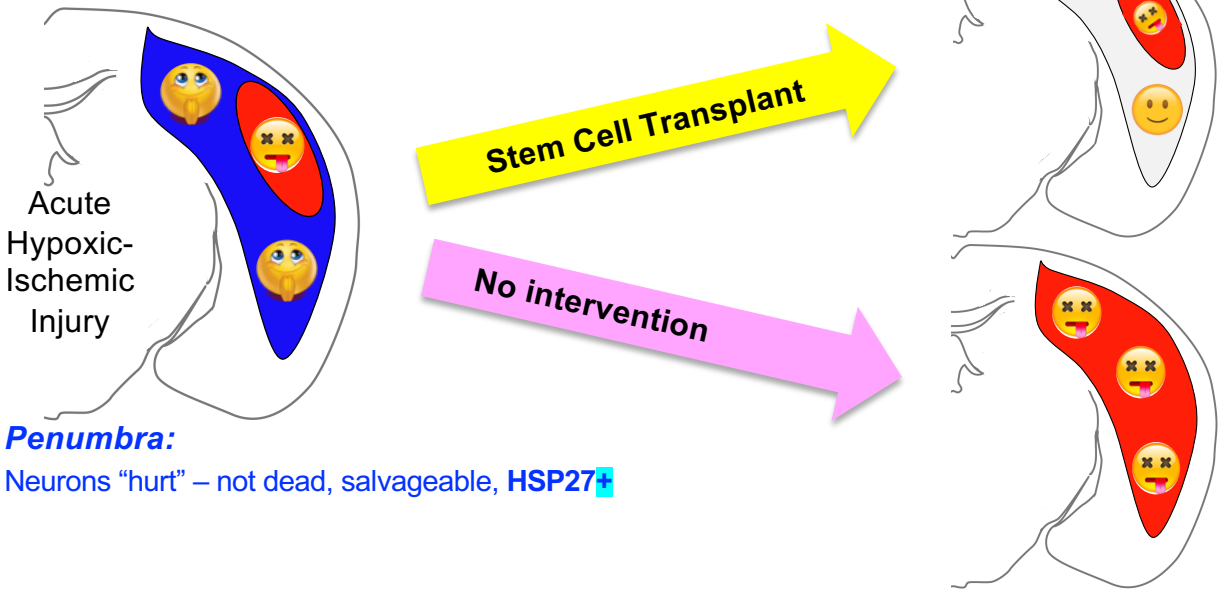
[9]Lead Contact
*Correspondence: esnyder@sbp.edu (E.Y.S.), sashwal@llu.edu (S.A.)
https://doi.org/10.1016/j.celrep.2020.107622

Cell Reports *31*, 107622, May 12, 2020

- Why should we care?
- What's so critical about the penumbra?
- Why should that be impactful?
- How do we know important "stuff" "lives" there?

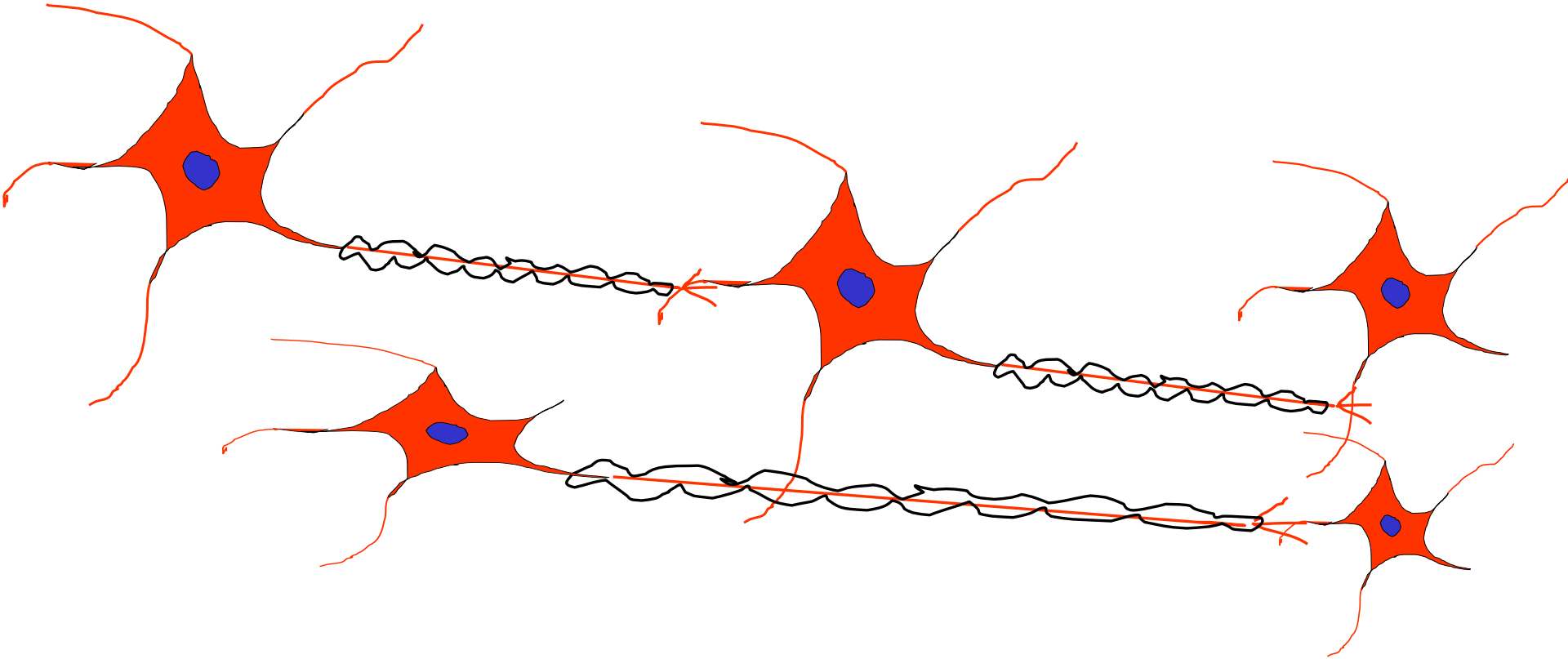
Core:

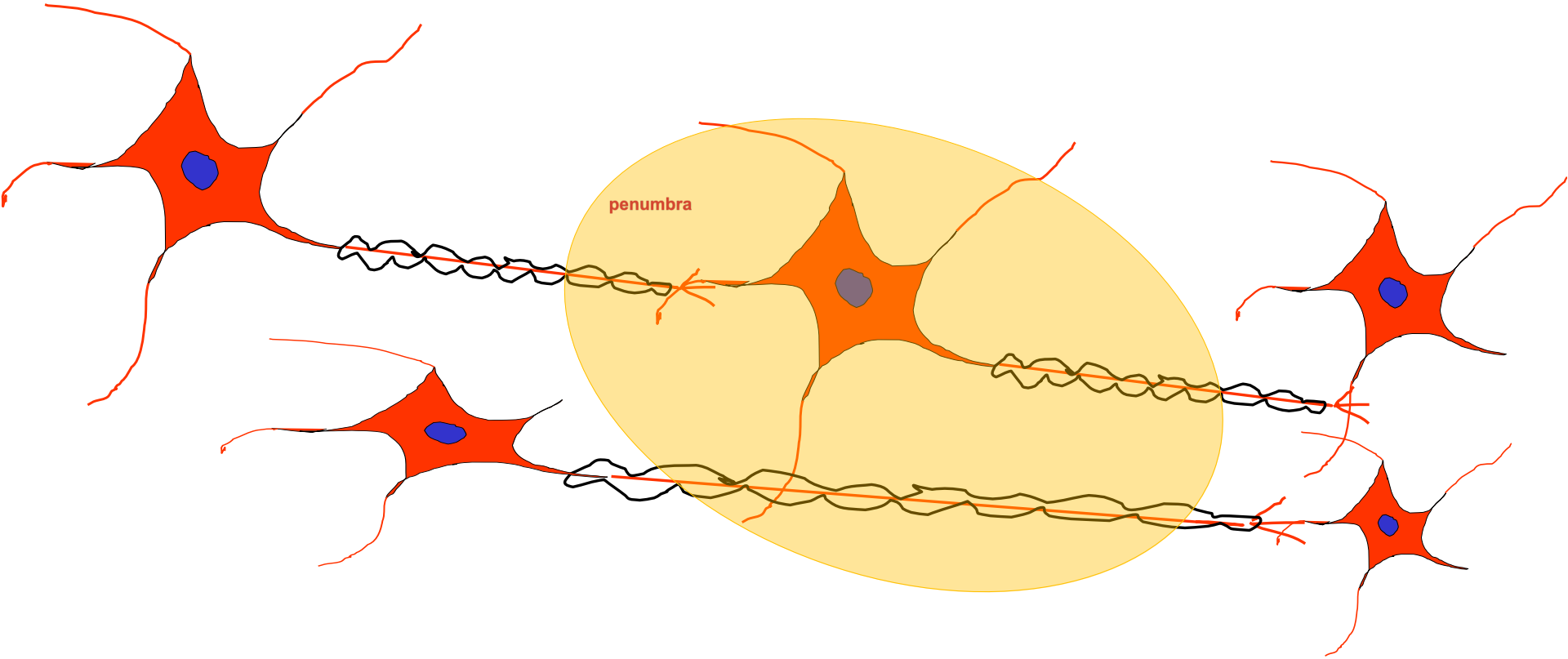
Neurons dead, unsalvageable, **HSP27–**



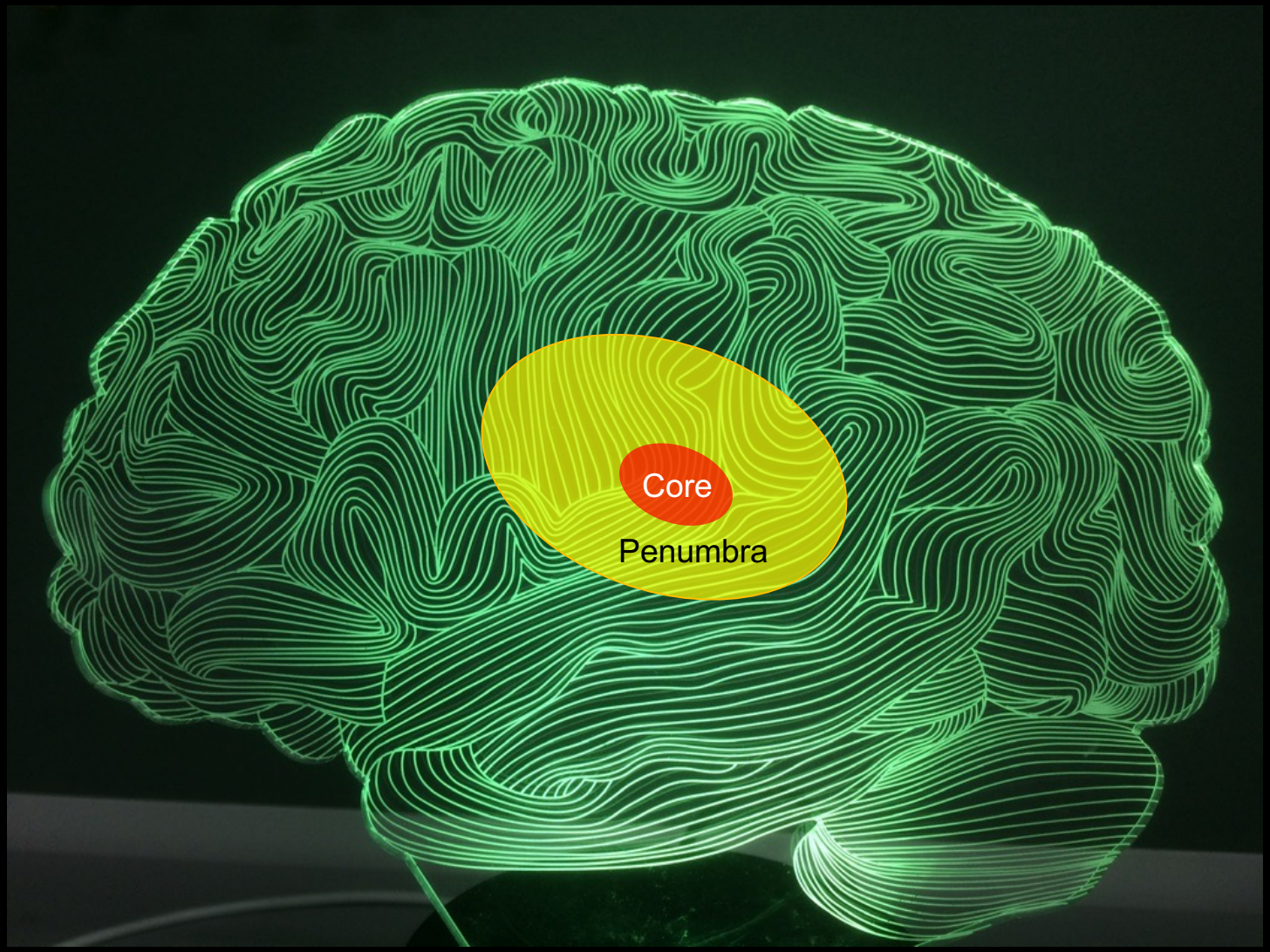
Penumbra:

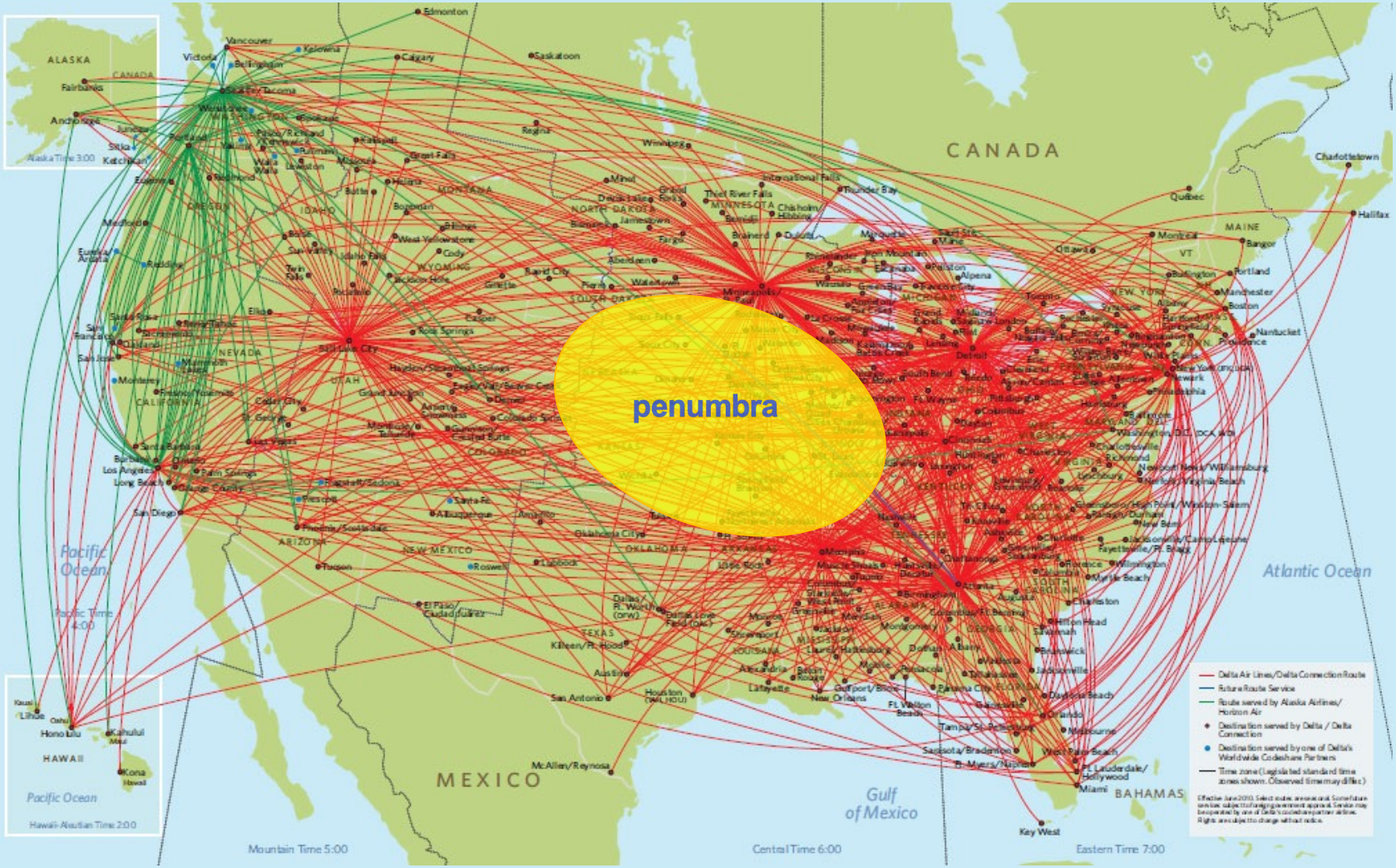
Neurons "hurt" – not dead, salvageable, **HSP27+**



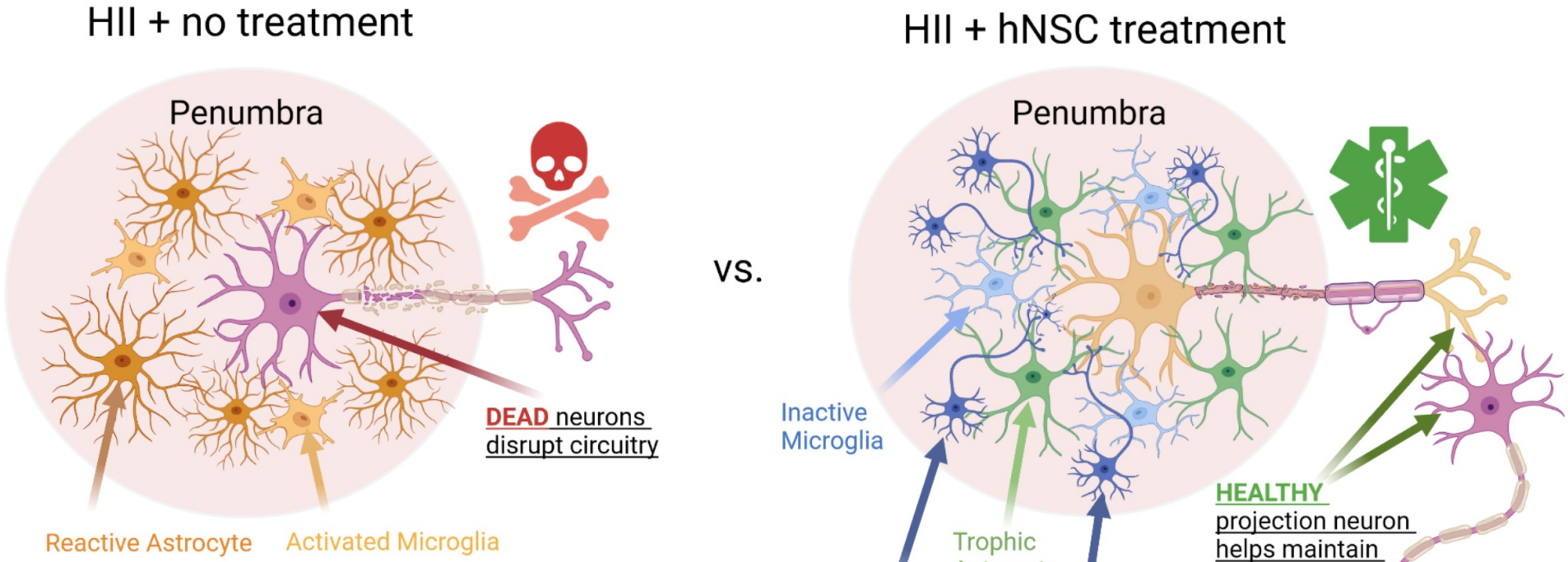


Fibres de passage


Core
Penumbra


penumbra
CANADA
MEXICO
ALASKA
HAWAII
Pacific Ocean
Atlantic Ocean
Gulf of Mexico
BAHAMAS
Mountain Time 5:00
Central Time 6:00
Eastern Time 7:00
Pacific Time 4:00
Alaska Time 3:00
Hawaii-Aleutian Time 2:00
Delta Air Lines/Delta Connection Route
Future Route Service
Route served by Alaska Airlines/ Horizon Air
Destination served by Delta / Delta Connection
Destination served by one of Delta's Worldwide Codeshare Partners
Time zone (Legislated standard time zones shown. Observed time may differ.)

Working Model & Hypothesis



?Preserving *fibres de passage* from axotomy & degeneration within the penumbra is mediated by a fate shift in astrocytes back to "trophic" *rather than* reactive – induced by donor fetal hNSCs

Rus Nuryyev

PUTATIVE MECHANISMS-OF-ACTION

(most of which are simply constitutive expressions of the NSC's fundamental homeostatic, physiological role)

- Direct neuroprotection & trophic support via *diffusible factors, gap junctions, exosomes*
  - (e.g., cytokines such as GDNF, BDNF, NT-3, NT-4, NGF, Nurturin)
- Scavenging ROS & excitoxins
- Promoting angiogenesis
- Mobilizing endogenous NSCs
- Replacing interneurons
- Altering niche
- Glial support
  - e.g., astrocytes & myelinating & non-myelinating oligodendrocytes
- Inducing neural self-repair, known to occur in injured immature newborn mammalian brain
- ↓ inflammation & scarring
- Repairing the blood-brain barrier
- Promoting endogenous neurite outgrowth
- Providing extracellular matrix
- Restoring normal metabolism to injured host cells

Cell Reports

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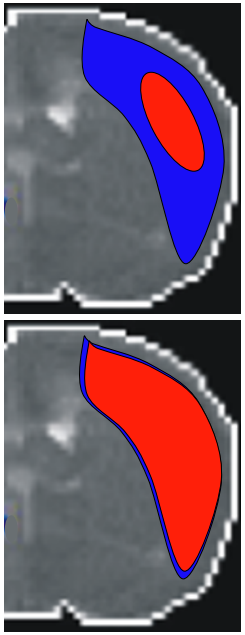
Report

A Biomarker for Predicting Responsiveness to Stem Cell Therapy Based on Mechanism-of-Action: Evidence from Cerebral Injury

Clinical Implications:

Arguably regenerative medicine's 1st "biomarker" for patient stratification:



***Consider* transplantation**

Penumbra > Core:

Regions potentially responsive to cell-based neuroprotection predominate

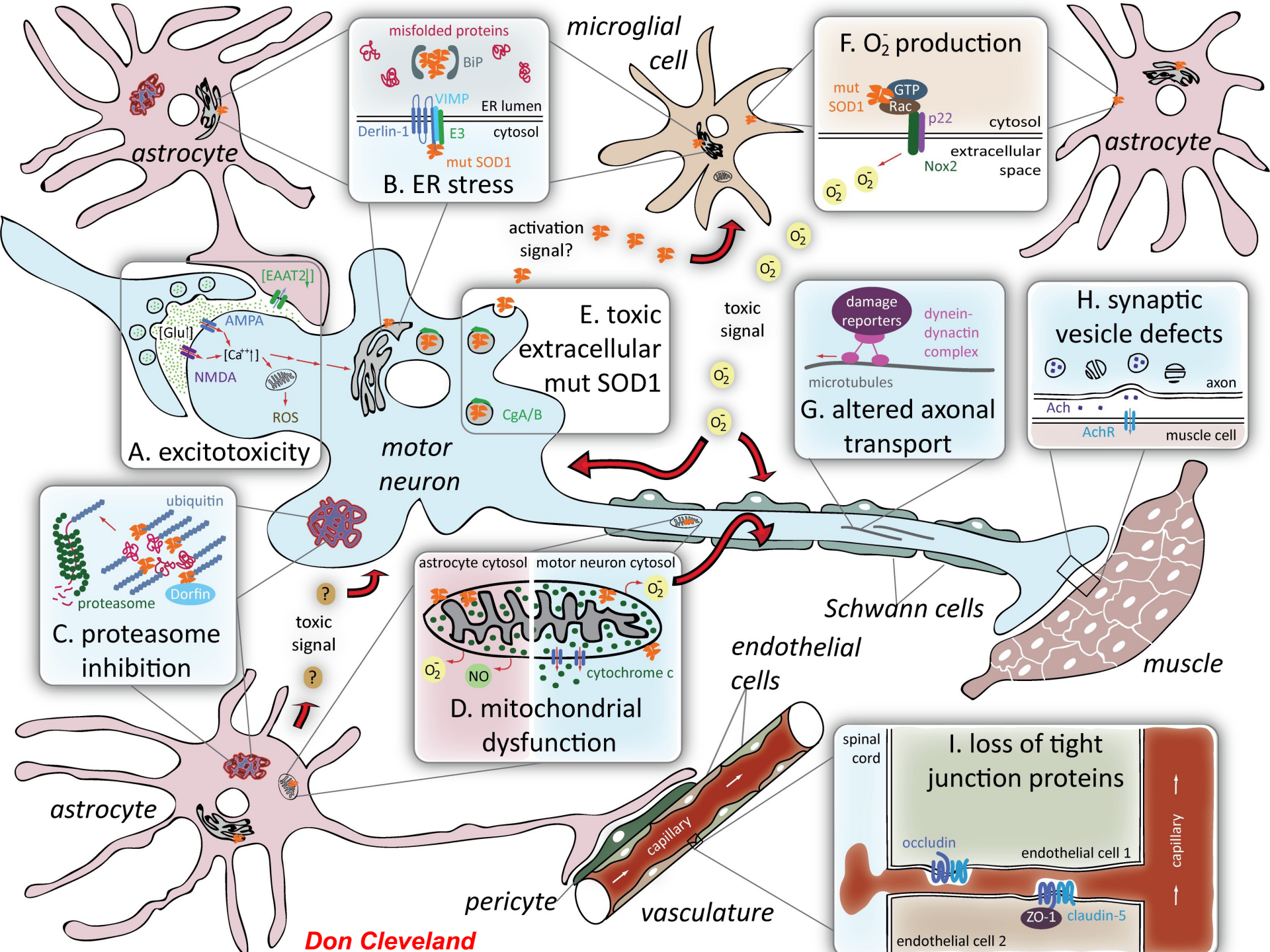
Transplantation inappropriate

Core > Penumbra:

Non-responsive regions predominate

isease
lerosis
ALS])

The pathophysiology of motor neuron degeneration *(e.g., ALS)* is coming to be recognized as complex & multi-faceted…


astrocyte
misfolded proteins
BiP
VIMP
ER lumen
Derlin-1
E3
cytosol
mut SOD1
B. ER stress
microglial cell
F. O_2^- production
mut SOD1
GTP
Rac
p22
cytosol
extracellular space
Nox2
O_2^-
astrocyte
activation signal?
[EAAT2]
[Glu]
AMPA
$[Ca^{++}]$
NMDA
ROS
A. excitotoxicity
motor neuron
E. toxic extracellular mut SOD1
CgA/B
toxic signal
damage reporters
dynein-dynactin complex
microtubules
G. altered axonal transport
H. synaptic vesicle defects
axon
Ach
AchR
muscle cell
ubiquitin
proteasome
Dorfin
C. proteasome inhibition
astrocyte cytosol
motor neuron cytosol
cytochrome c
NO
D. mitochondrial dysfunction
Schwann cells
muscle
endothelial cells
astrocyte
pericyte
capillary
vasculature
spinal cord
I. loss of tight junction proteins
occludin
endothelial cell 1
ZO-1
claudin-5
endothelial cell 2
capillary
Don Cleveland

…suggesting that some aspects may be well-suited (at least in part) for the multi-faceted actions of the stem cell

- Could there be a “mapping” of an NSC action to a particular pathophysiological process in the SOD1 mouse model of ALS?

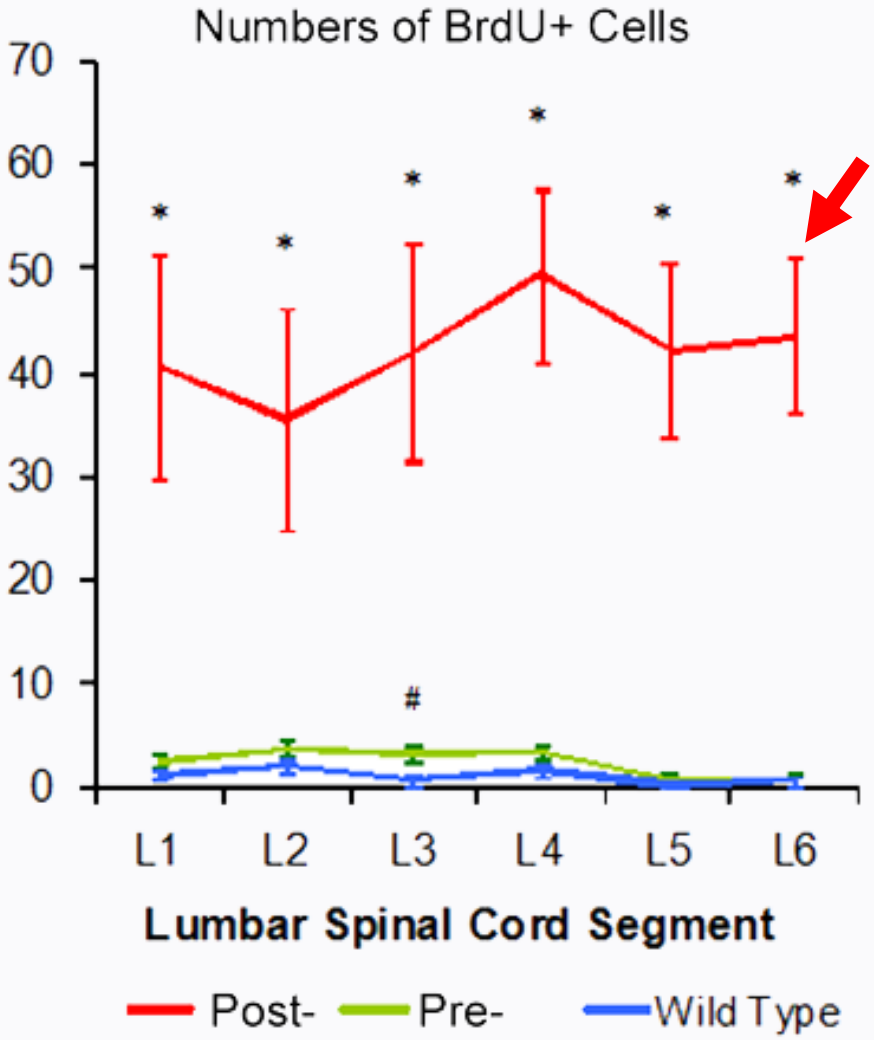
The constitutive dynamics of the *endogenous* progenitor cells in ALS are *different* from that in HII

Dynamics of Endogenous Cells in SOD1 Mouse Model of ALS

- Most prominent BrdU incorporation in *rapidly progressing mice*

- BrdU+ cells = *astroglia*
  - Bear mutant *SOD1*
  - Toxic / non-trophic / non-protective

- **To restore homeostasis:**
  - Suppress emergence/proliferation of endogenous mutant toxic & reactive astrocytes
  - supply "replacement" *non*-mutant trophic astrocytes
  - Restore non-toxic milieu



Teng et al, Science Translational Medicine (2012)

Science Translational Medicine
AAAS

- 11 studies across 3 centers
- Same undifferentiated multipotent migratory CNS-derived NSCs in in same colony of SOD1^{G93A} mice
- Early affected adult
- Administered intra-parenchymally / intra-central canal using
- Same SOP
- 4 key loci along neuraxis subserving life-sustaining functions

Multimodal Actions of Neural Stem Cells in a Mouse Model of ALS: A Meta-Analysis

Yang D. Teng et al.
Sci Transl Med **4, 165ra164 (2012);**
DOI: 10.1126/scitranslmed.3004579

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Jeffrey D. Rothstein
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Evan Y. Snyder

Ted Teng

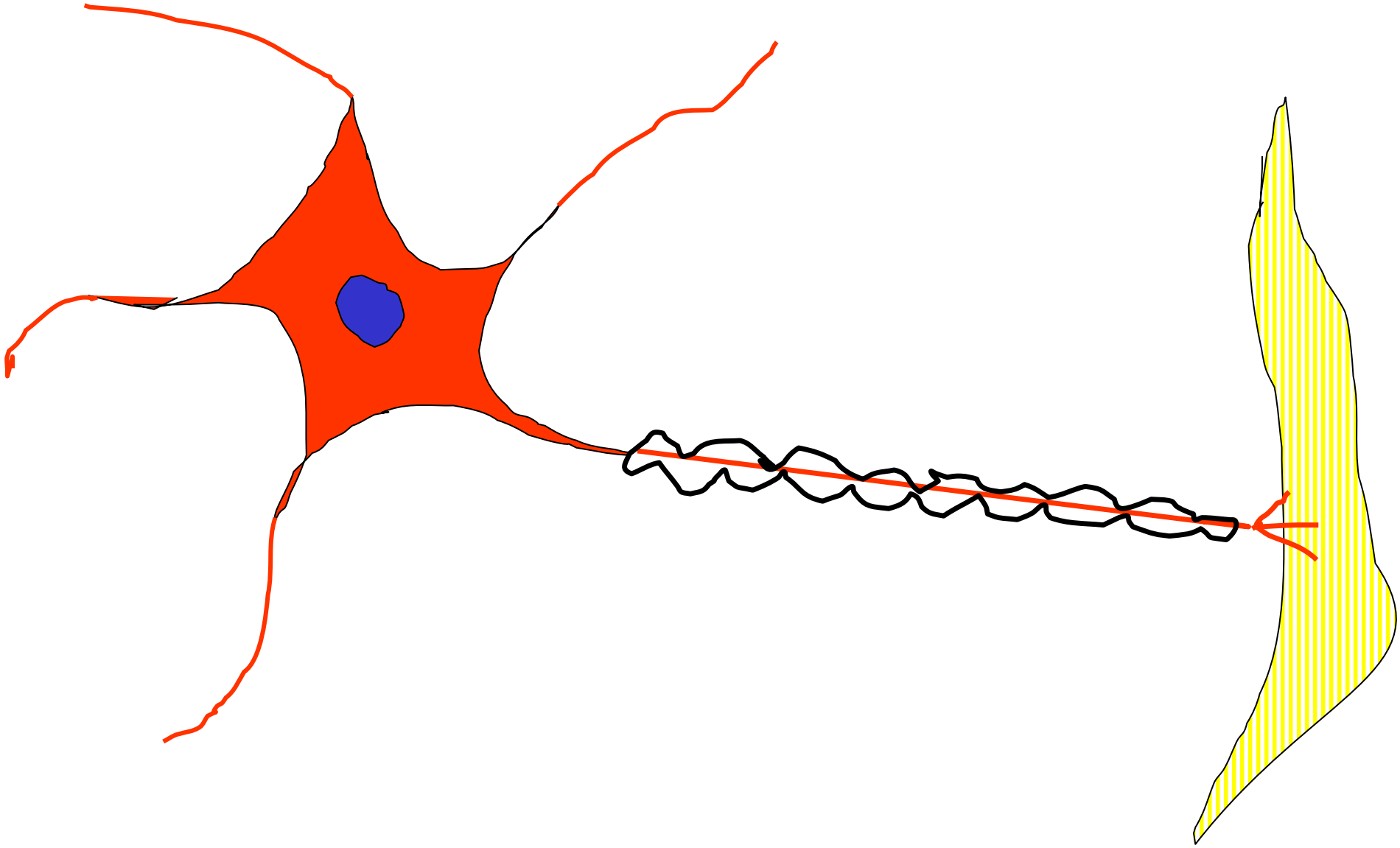
Representative SOD1^{G39A} Transgenic Mouse Model of ALS Treated with hNSCs

- Delayed disease-onset
- Slowed disease progression
- Improved Motor performance

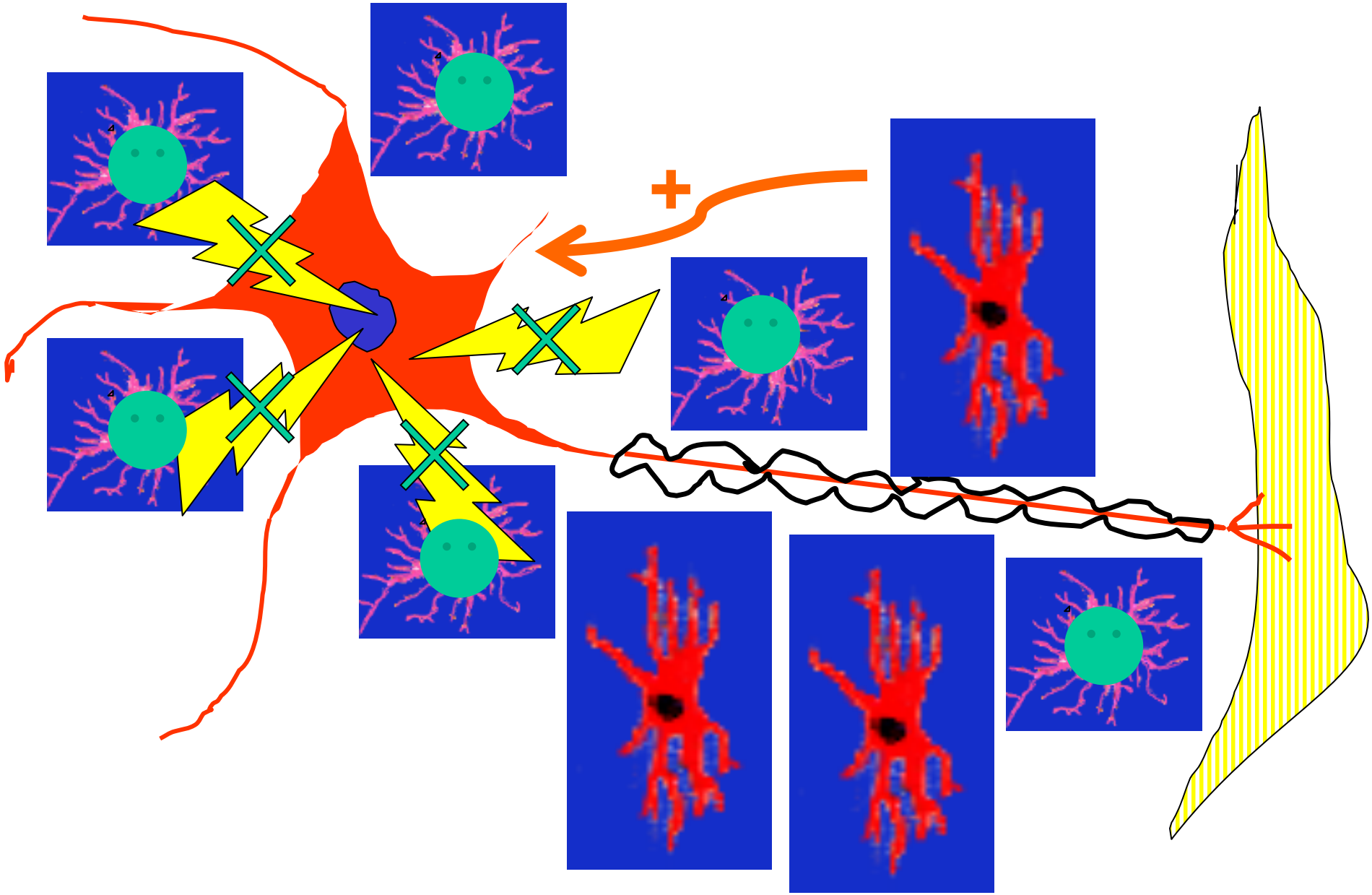
Science Translational Medicine AAAS

Multimodal Actions of Neural Stem Cells in a Mouse Model of ALS: A Meta-Analysis
Teng et al, STM **4, 165ra164 (2012);**

Ted Teng



Teng et al, Science Translational Medicine (2012)



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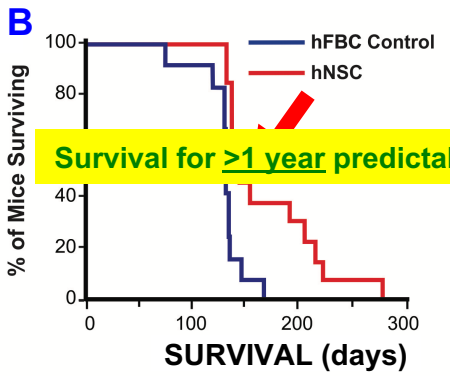
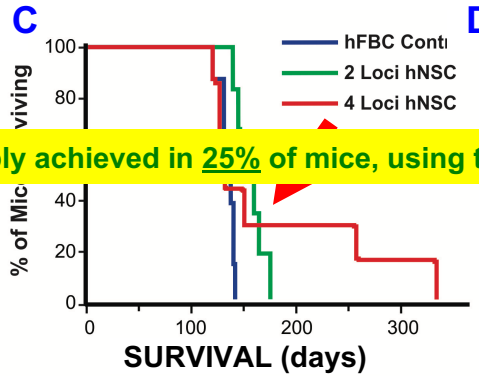
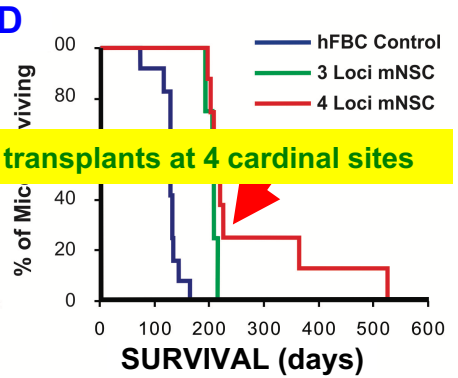
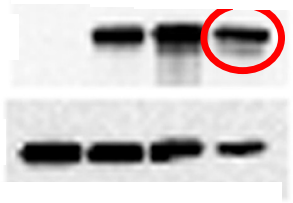
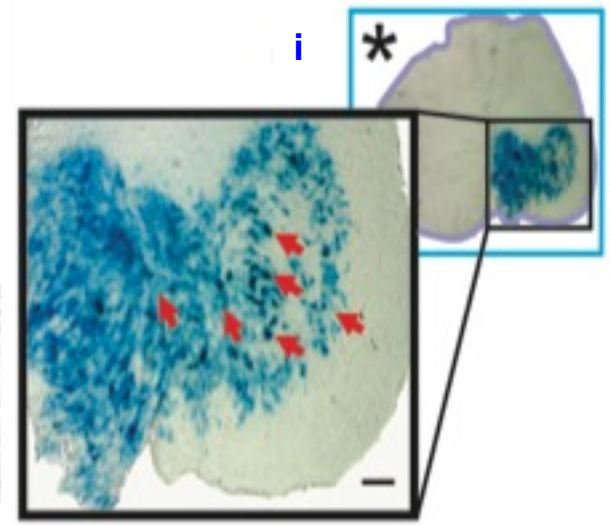


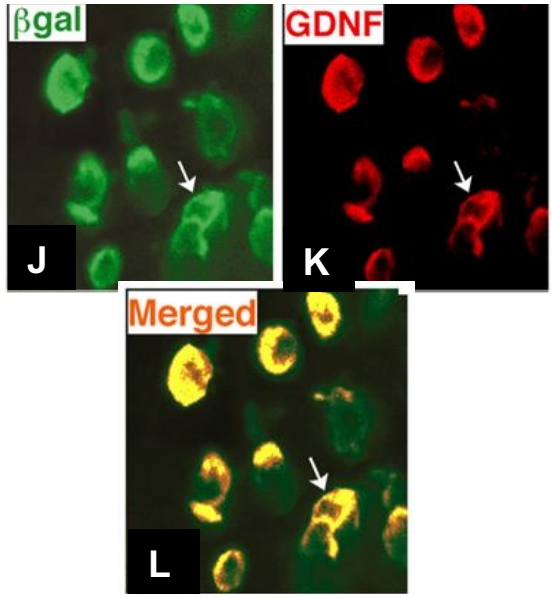




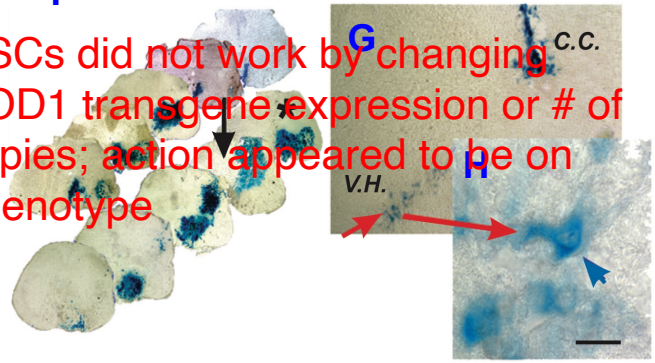
Survival for >1 year predictably achieved in 25% of mice, using transplants at 4 cardinal sites

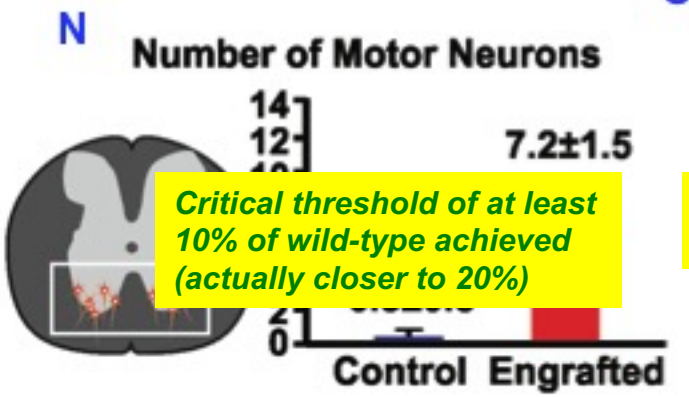




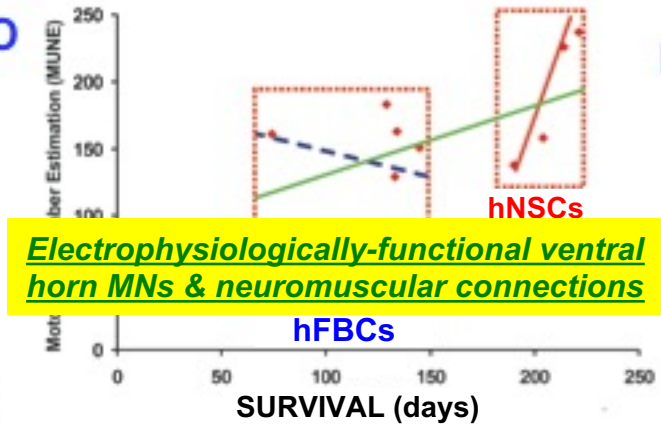


NSCs did not work by changing SOD1 transgene expression or # of copies; action appeared to be on phenotype

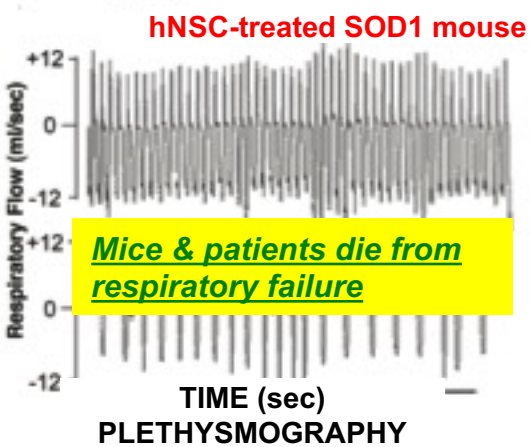




Critical threshold of at least 10% of wild-type achieved (actually closer to 20%)

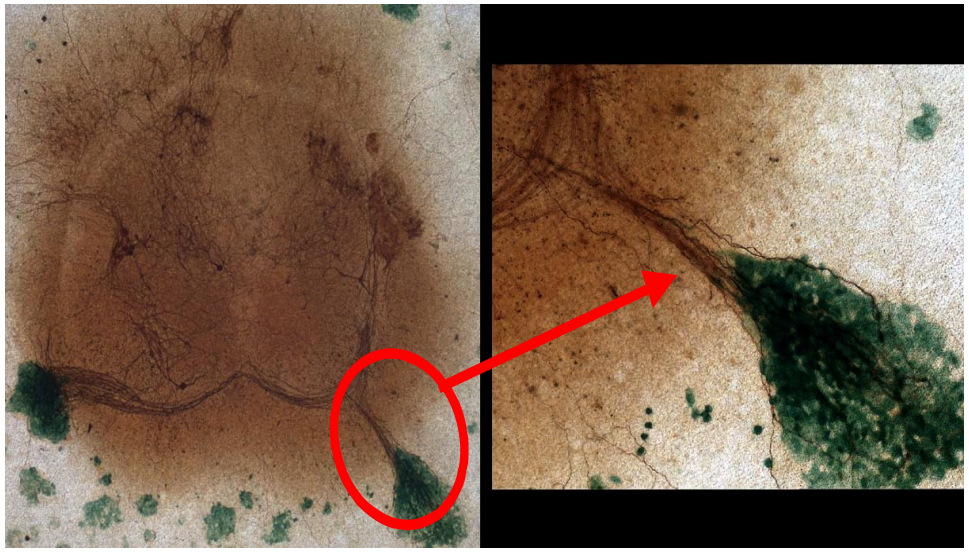


Electrophysiologically-functional ventral horn MNs & neuromuscular connections

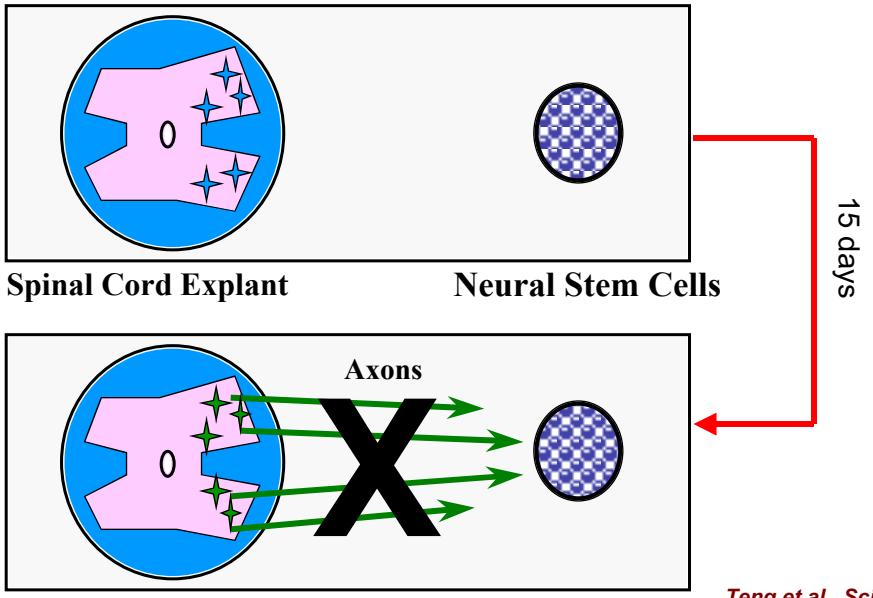


Mice & patients die from respiratory failure

Assay to determine whether NSCs produce *functional* trophic agents (of which GDNF is likely just one): *Induction by NSCs of spinal ventral horn motor neuron axonal outgrowth*

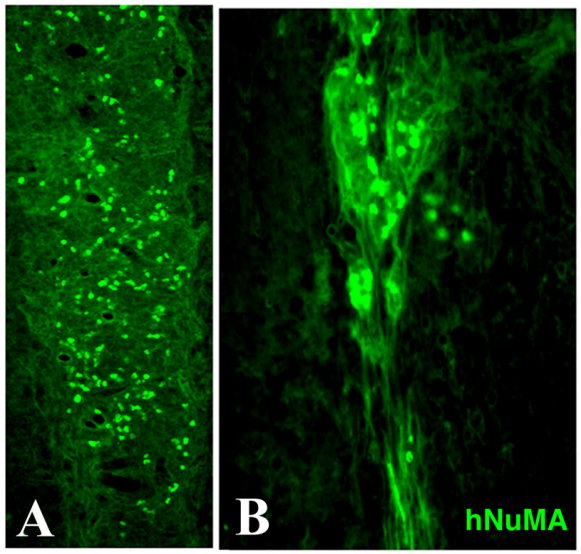


Spinal Cord Organotypic Culture

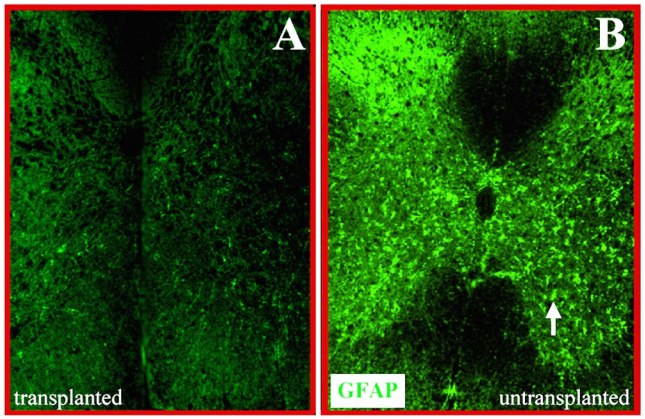


- **Replicated by** exogenous GDNF
- **BLOCKED BY**
  - GDNF Anti-Sense
  - GDNF Soluble Receptor
  - Differentiation of NSCs into neurons
  - Spinal cord slices from *Ret* KO mouse

Teng et al, Science Translational Medicine (2012)



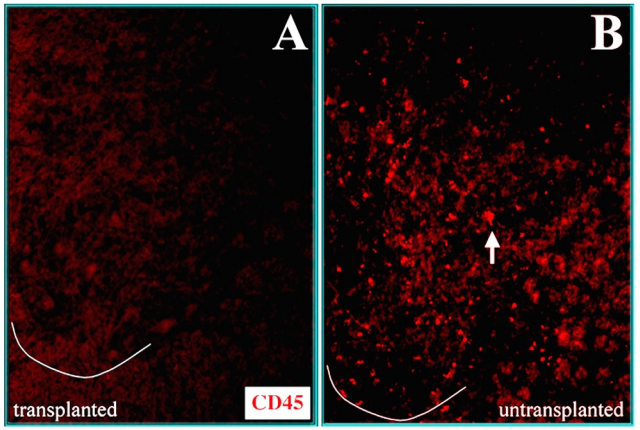
II.



↓ Astrogliosis in hNSC-Tx'd SOD1 mice *(cervical region)*

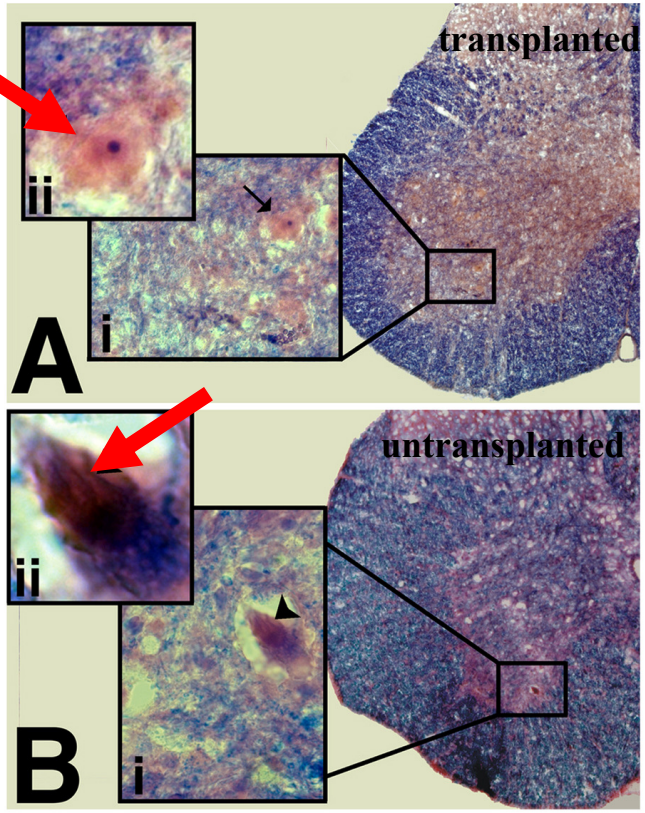
284 ± 13 (un-Tx'd) *vs* 178 ± 10 (Tx'd) per 20 µm cord (p < 0.001)

III.



↓ Macrophage / Microglial Infiltration in hNSC-Tx'd SOD1 Mice

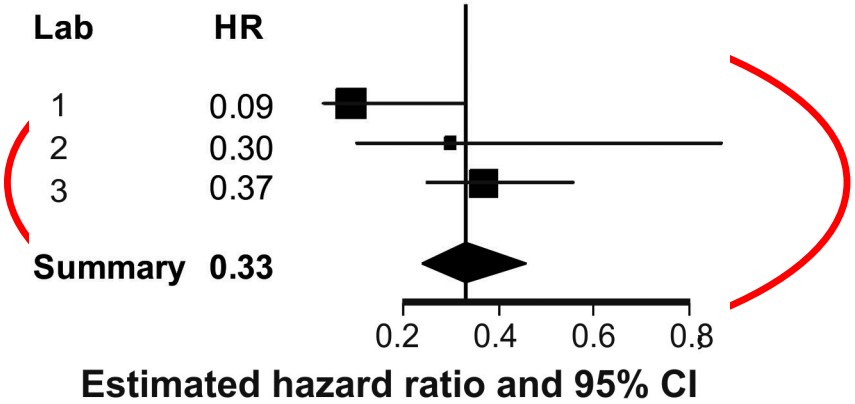
153 ± 7 (un-Tx'd) *vs* 9 ± 2 (Tx'd) per 20 µm cord (p < 0.001)



↓ Intraneuronal Neurofibrillary Tangles in NSC-Tx'd SOD1 mice

11 ± 2 (un-Tx'd) *Vs* 3 ± 0.8 (Tx'd) per 20 µm cord (p < 0.001)

V.



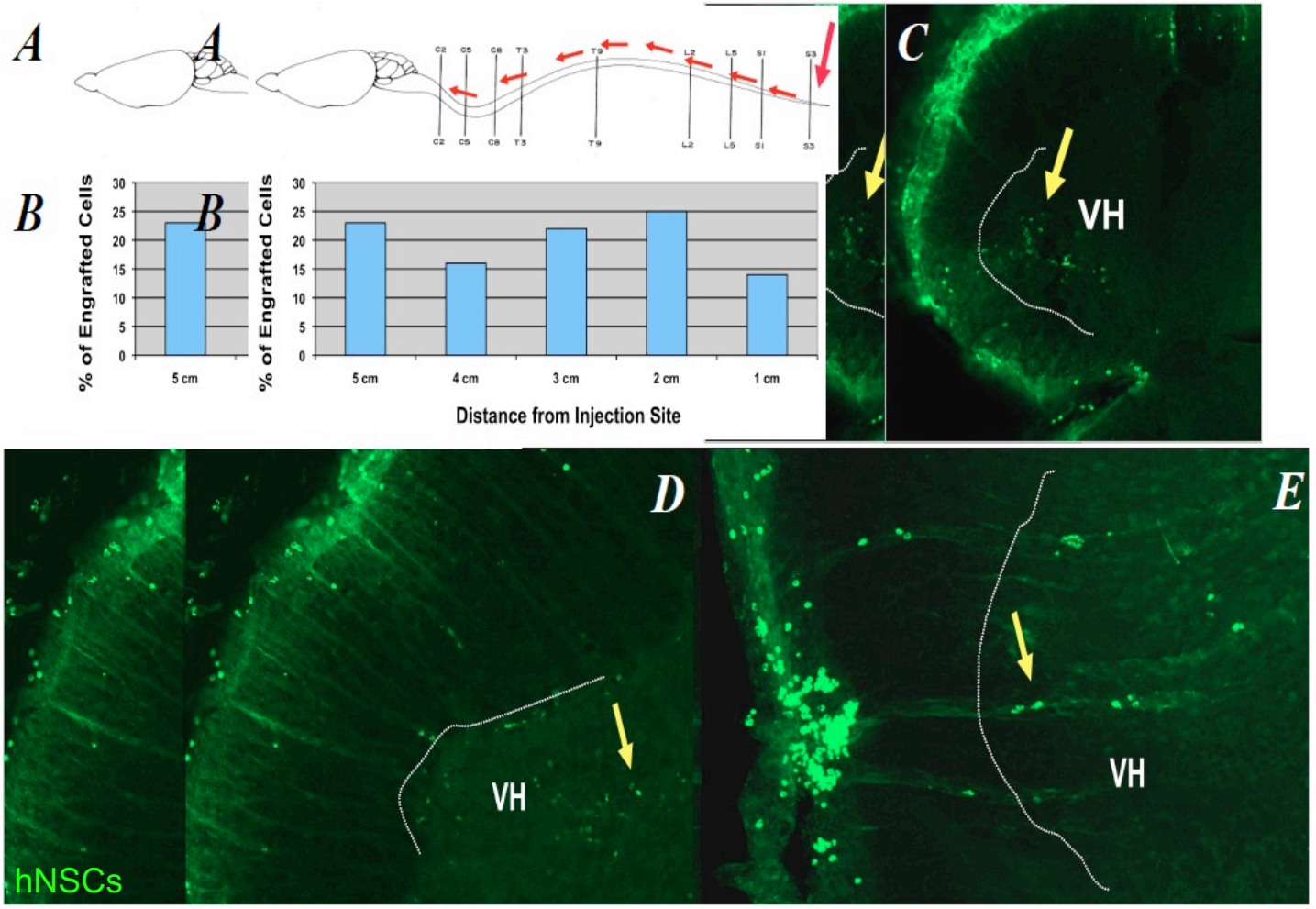
Meta-analysis (11 studies)

1 unit increase in # of injection foci associated with 30% decrease in hazard rate (HR=0.70; 95% CI: 0.62-0.79), suggesting broader range of donor

Hazard (i.e., risk of death) ratio = 0.33 (95% CI: 0.24-0.46)

Indicates NSC transplantation associated with a decrease in hazard rate of 67% compared with control

hNSCs can access ventral horn from intrathecal space if they track along ventral roots

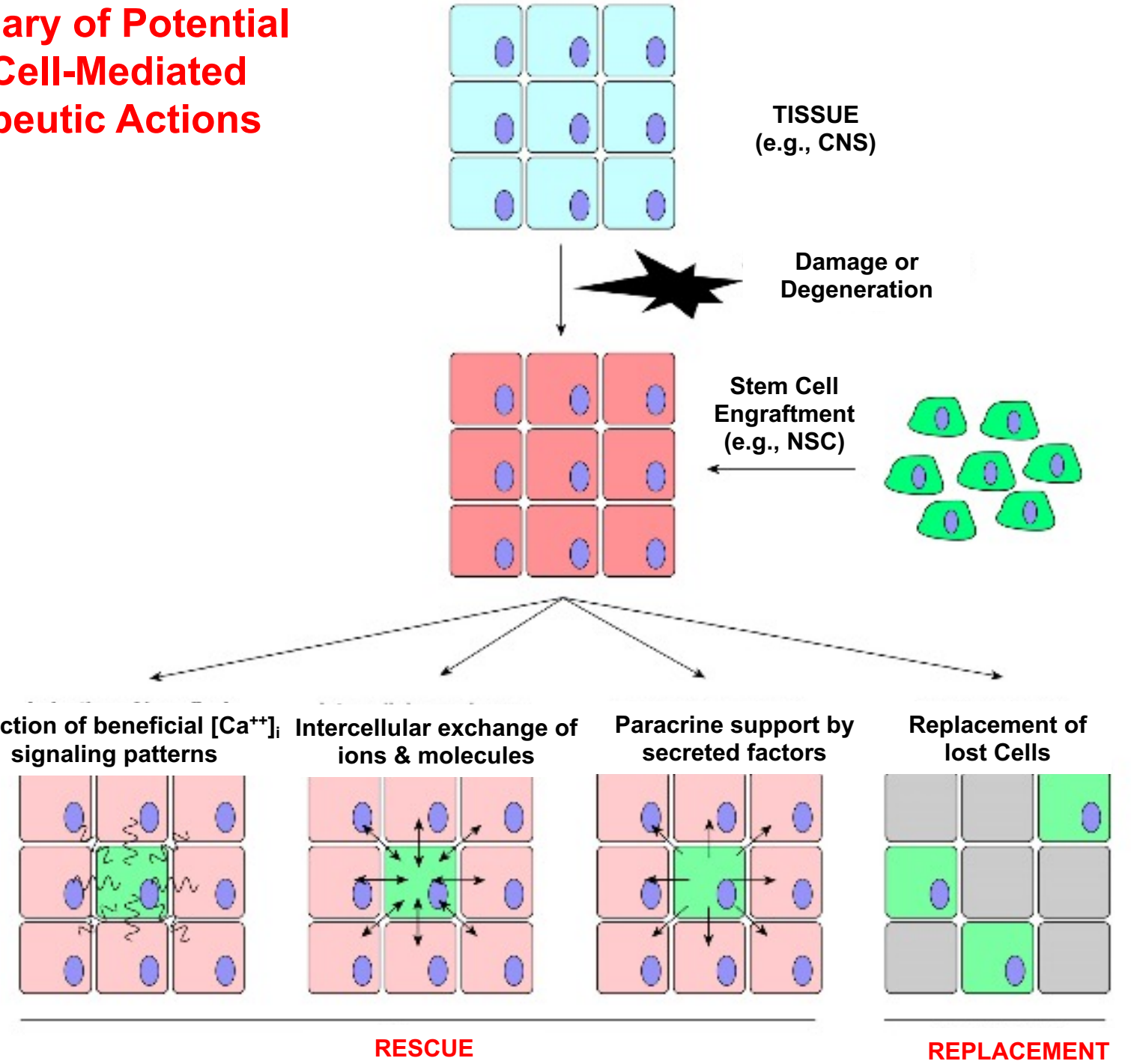


Doug Kerr

- Most diseases & injuries – particularly neurological – are *not* driven by single pathophysiological processes or involve single cell types
  (even if a particular cell type seems predominantly effected)
- ALS = a case-in-point
  - The pathophysiology of motor neuron degeneration is complex & multi-faceted
  - Strategies that attack multiple pathogenic processes are more likely to be successful than those that target just one
  - Growing recognition of multi-faceted actions of a true stem cell (particularly the NSC) simply by virtue of its fulfillment of its teleological developmental homeostasis-maintaining role
    - Could there be a "mapping" of an NSC action to a particular pathophysiological process in SOD1 mouse model of ALS?
- "Cell replacement" in the nervous system means more than replacing "*neurons*" –
  - Glia?
  - Microglia?
  - Vascular endothelial cells?
  - Vascular smooth muscle?

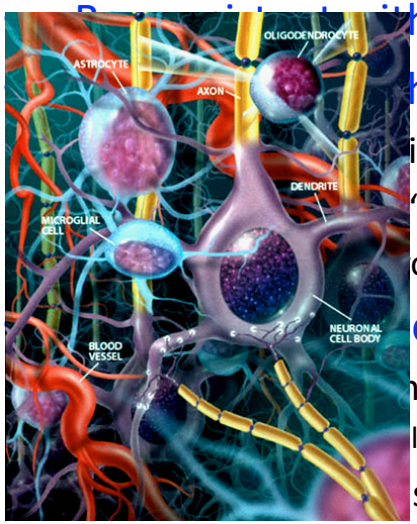
- ALS / (?SMA) (*Science Trans Med, 2012*)
- Parkinson's Disease (*Nat Biotech* '02; *PNAS* '07; *Stem Cells* '09)
- Neurogenetic degeneration (*Nat Med* '07; *Stem Cells* '09)
- Some aging-related degeneration
- Spinal cord injury & Head trauma (*PNAS* '02; PNAS'10)
- Stroke / Hypoxic-Ischemia (*Nat Biotech* '02; *Exp Neurol* '06; PNAS'11)
- Cerebellar Degeneration (*J Neurosci* '06; *PNAS* '06; PNAS'10)

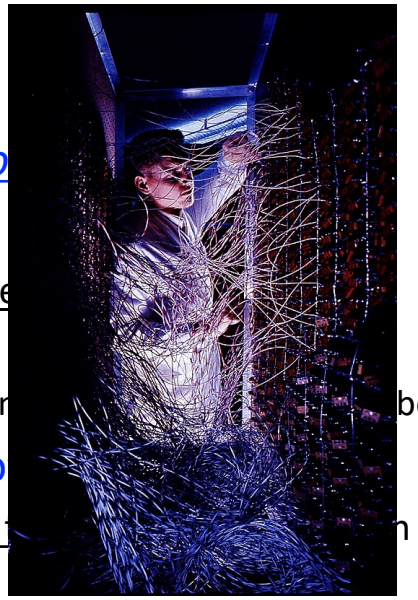
Summary of Potential Stem Cell-Mediated Therapeutic Actions



MY PRINCIPLES OF STEM CELL THERAPY FOR THE NERVOUS SYSTEM

- Make sure your cell can participate in normal developmental, functional, & homeostatic processes
  - "*Repair* strategies" may need to reinvoke "*developmental* strategies"
- Understand what you are treating
  - Protecting neural networks more tractable than reconstructing/replacing them (for that must know exquisite amounts of developmental biology)
    - Treat as early in disease process as possible
  - But also understand disease sufficiently to know *how* to protect





h biological imperatives of the organ's *homeo*

h biological imperatives of the *stem cell*

ing" it to do something that goes counter to those impe

'belongs" where you are going to put it

opriate reciprocal cross-talk to promote homeostasis may r berrant

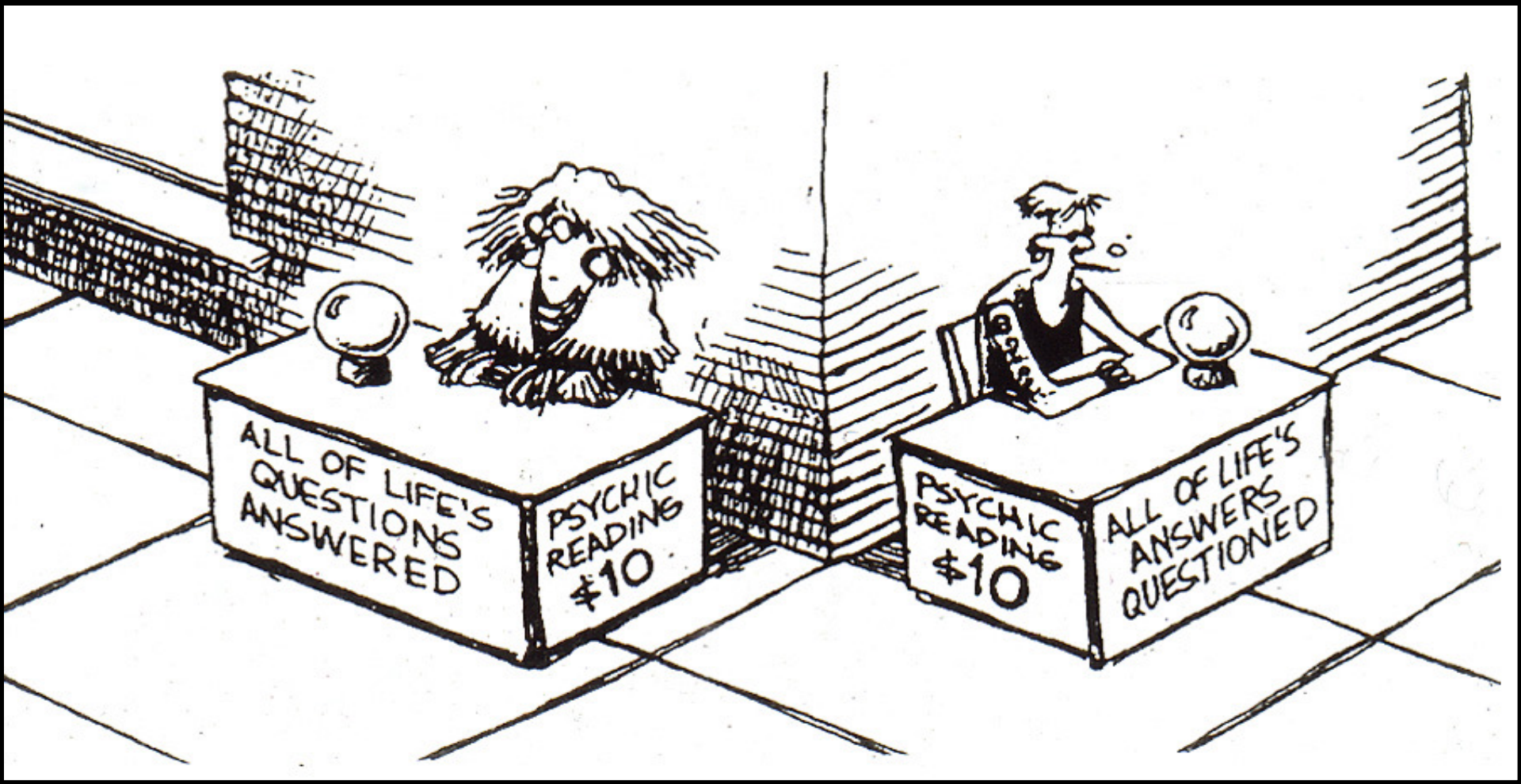
of pathogenic mechanism at play in disease-o

t possible if defect is cell autonomous or cell resistant n have

lantation or migration) to area where action needed

s for *products* of cell (must be able to reach target for requisite duration in requisite dose)

- When thinking about "regeneration/neuroprotection" be cognizant of "*all* lineages"
  - Many pathologic entities *injure multiple systems — not just neural*
    - e.g., stroke, trauma, infection, inflammation
  - Health or function of one cell may be dependent on another cell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