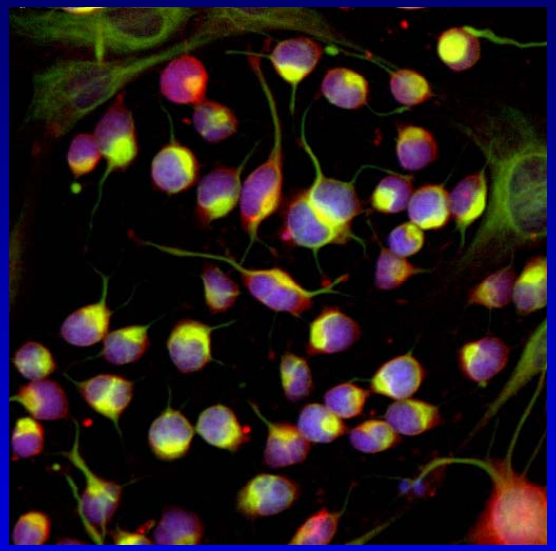
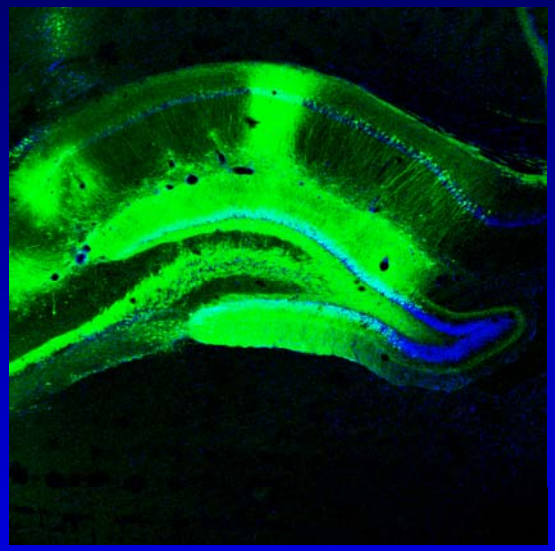
Stem Cells: Toward Engineering Therapies of the Future





David Schaffer, Ph.D.

Professor, Chemical Engineering, Bioengineering, & the Helen Wills Neuroscience Institute

Associate Director, Berkeley Stem Cell Center

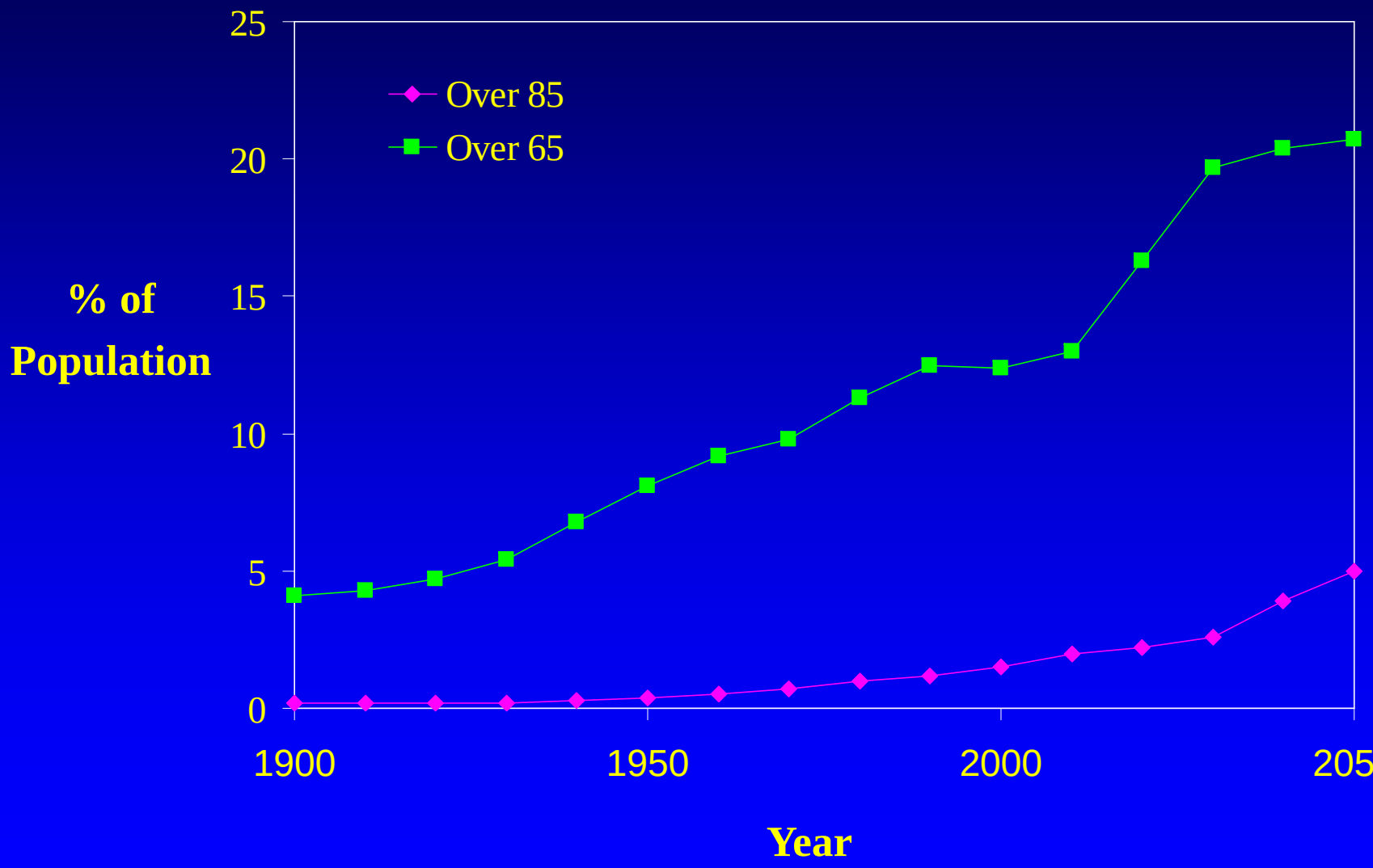
University of California at Berkeley

Historical Timeline in Molecular Medicine Development

Small Molecules

Protein Therapies

Trends in U.S. Demographics



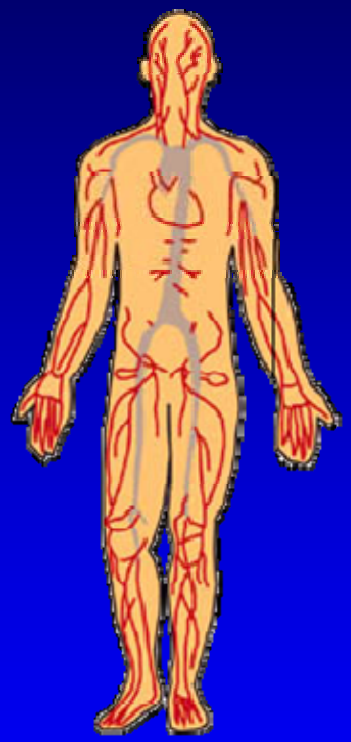
U.S. Census Bureau

Unmet Medical Needs in the Nervous System

Alzheimer's
Parkinson's
Huntington's
Spinocerebellar Ataxia
Stroke

Multiple Sclerosis
Injury

ALS (Lou Gehrig's)
Peripheral neuropathy



Retinitis pigmentosa
Macular degeneration
Glaucoma

Alzheimer's Disease:	4 million
Parkinson's Disease:	1.5 million
Stroke:	3-4 million

Historical Timeline in Molecular Medicine Development

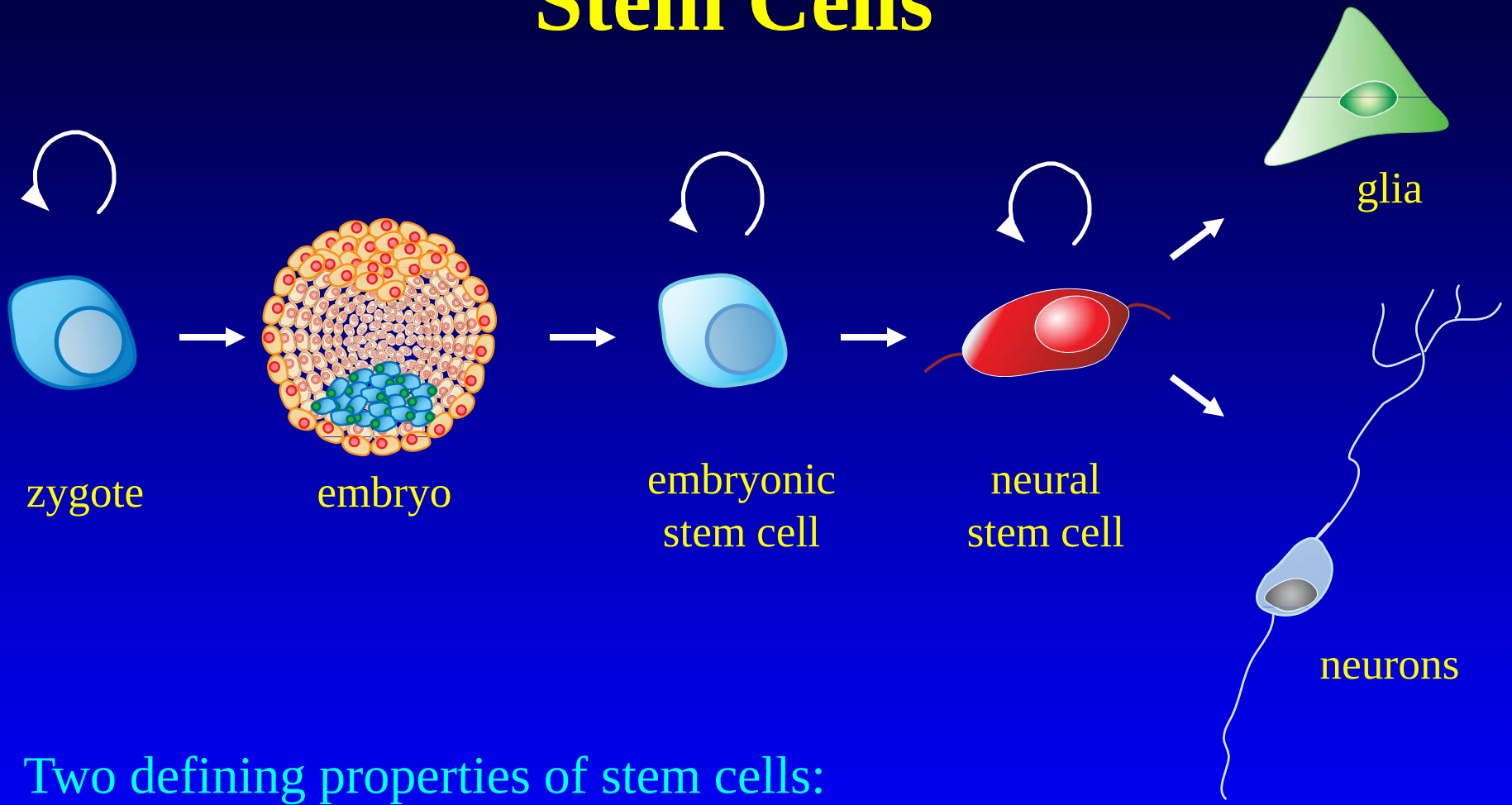
Small Molecules

Protein Therapies

Gene Therapy

Stem Cells

Stem Cells



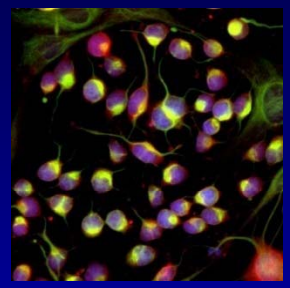
Two defining properties of stem cells:

1) self-renewal

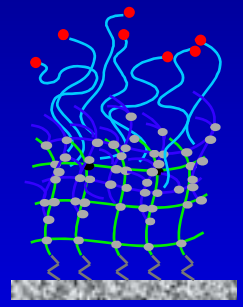
2) differentiation

Outline

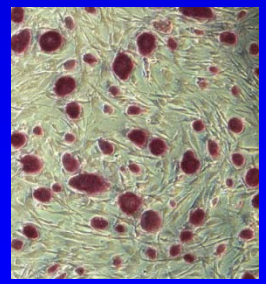
- Neural stem cells

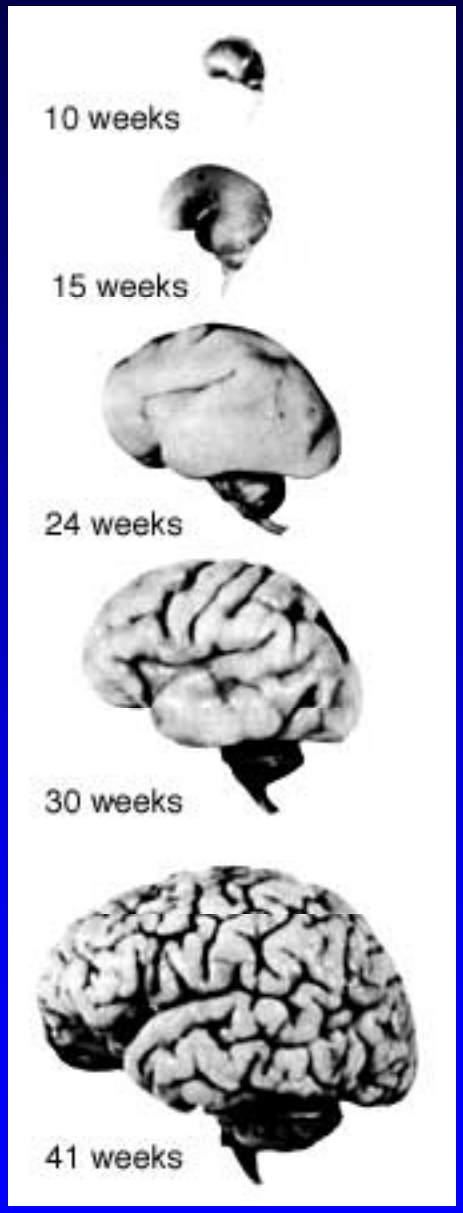


- Understanding and engineering the stem cell microenvironment



- Recent developments in the field



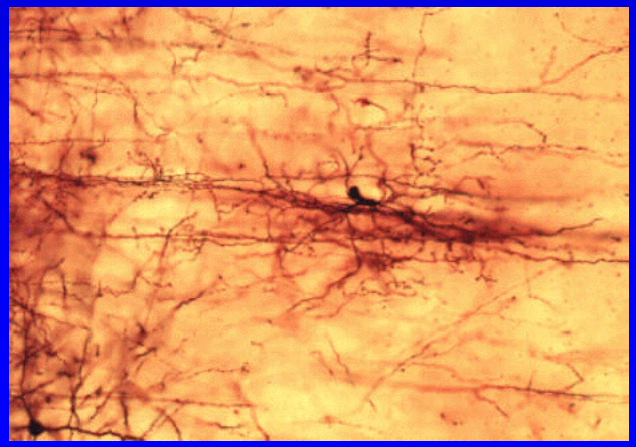


In humans, the largest number of neurons are born between 5 weeks and 5 months after conception (peak rate ~ 250,000 neurons/min)

Stem cells in the adult nervous system … ?

"Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree."

S. Ramon y Cajal, *Degeneration and regeneration of the nervous system*, 1928



Neurogenic Regions in the Adult Mammalian Brain

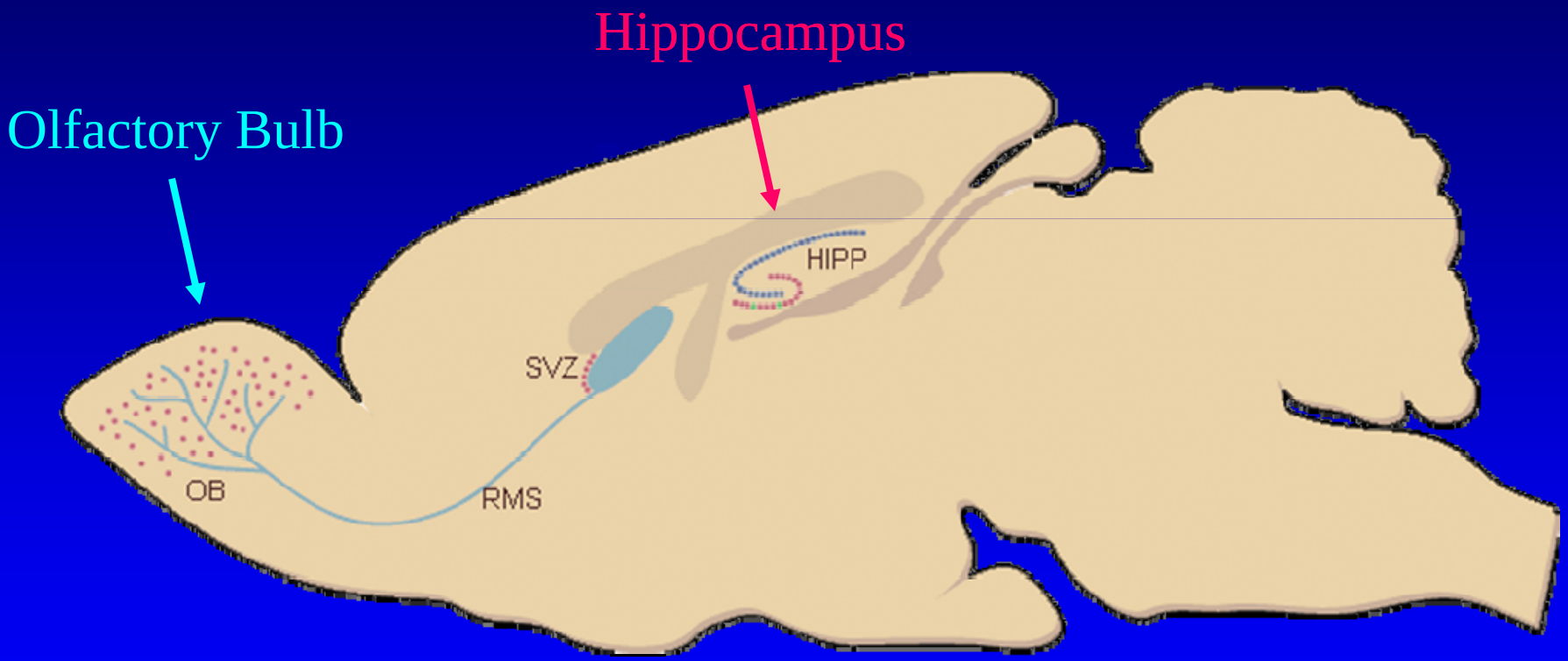
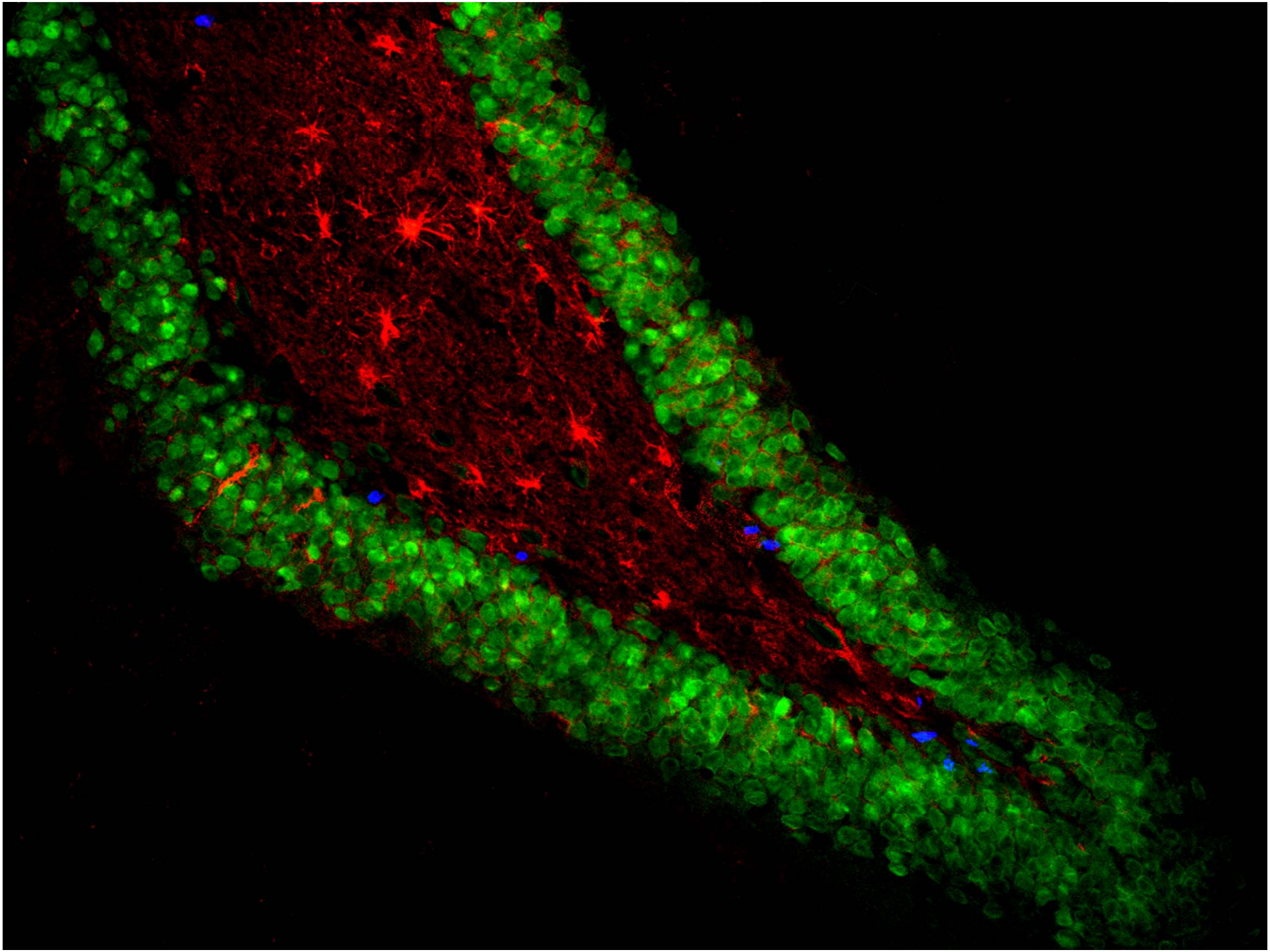
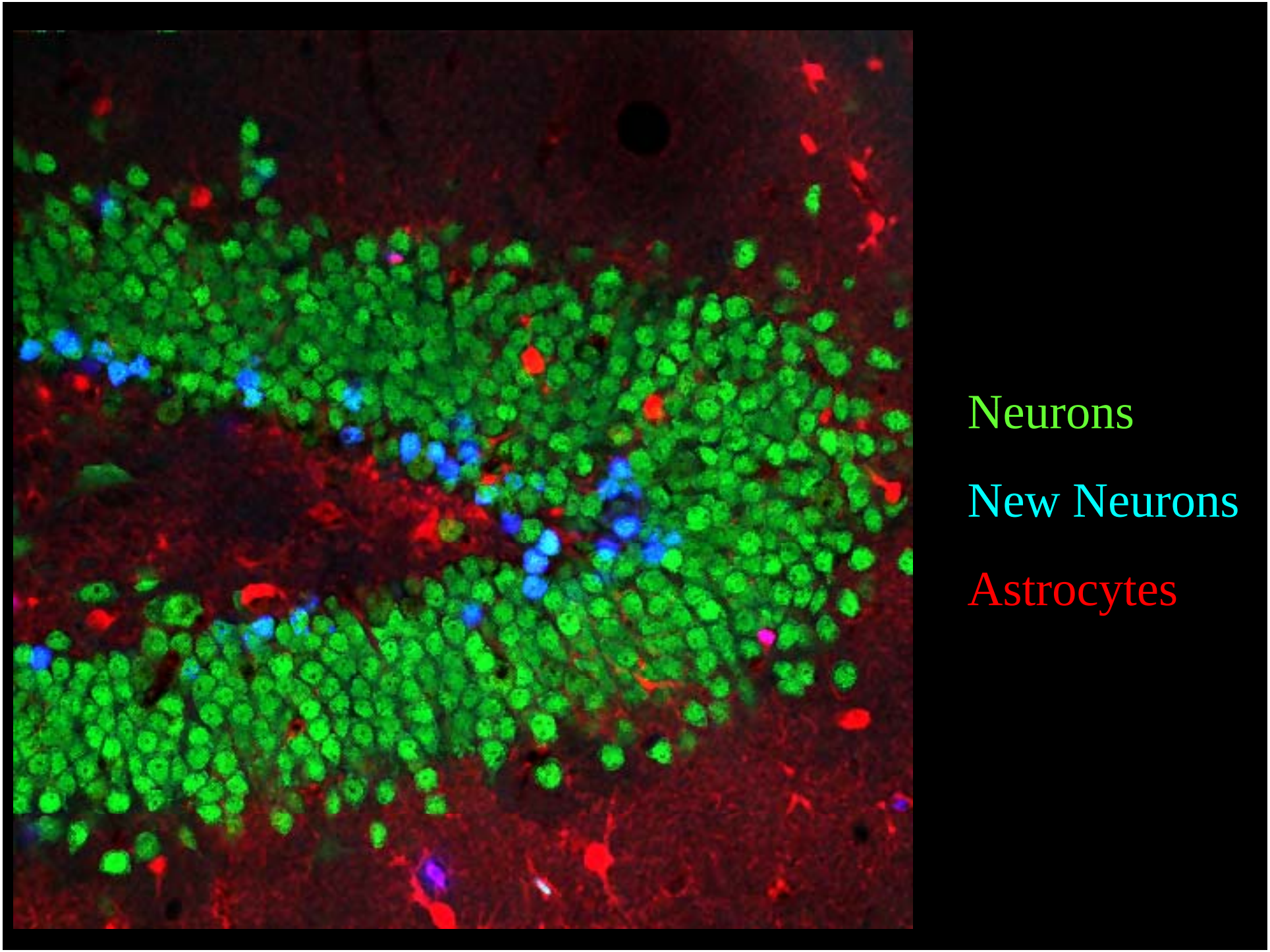
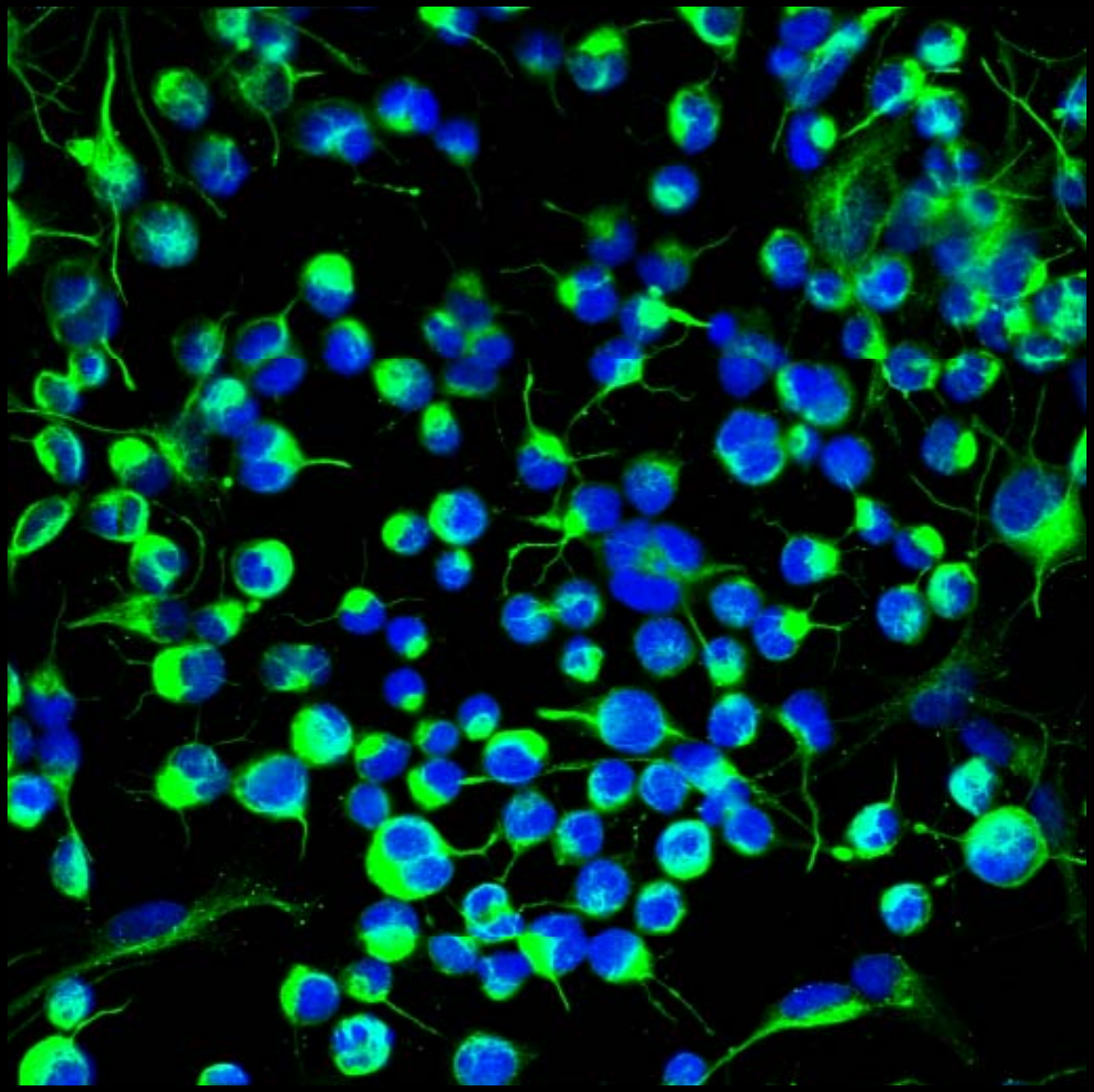


Image from www.scholarpedia.org




Neurons
New Neurons
Astrocytes

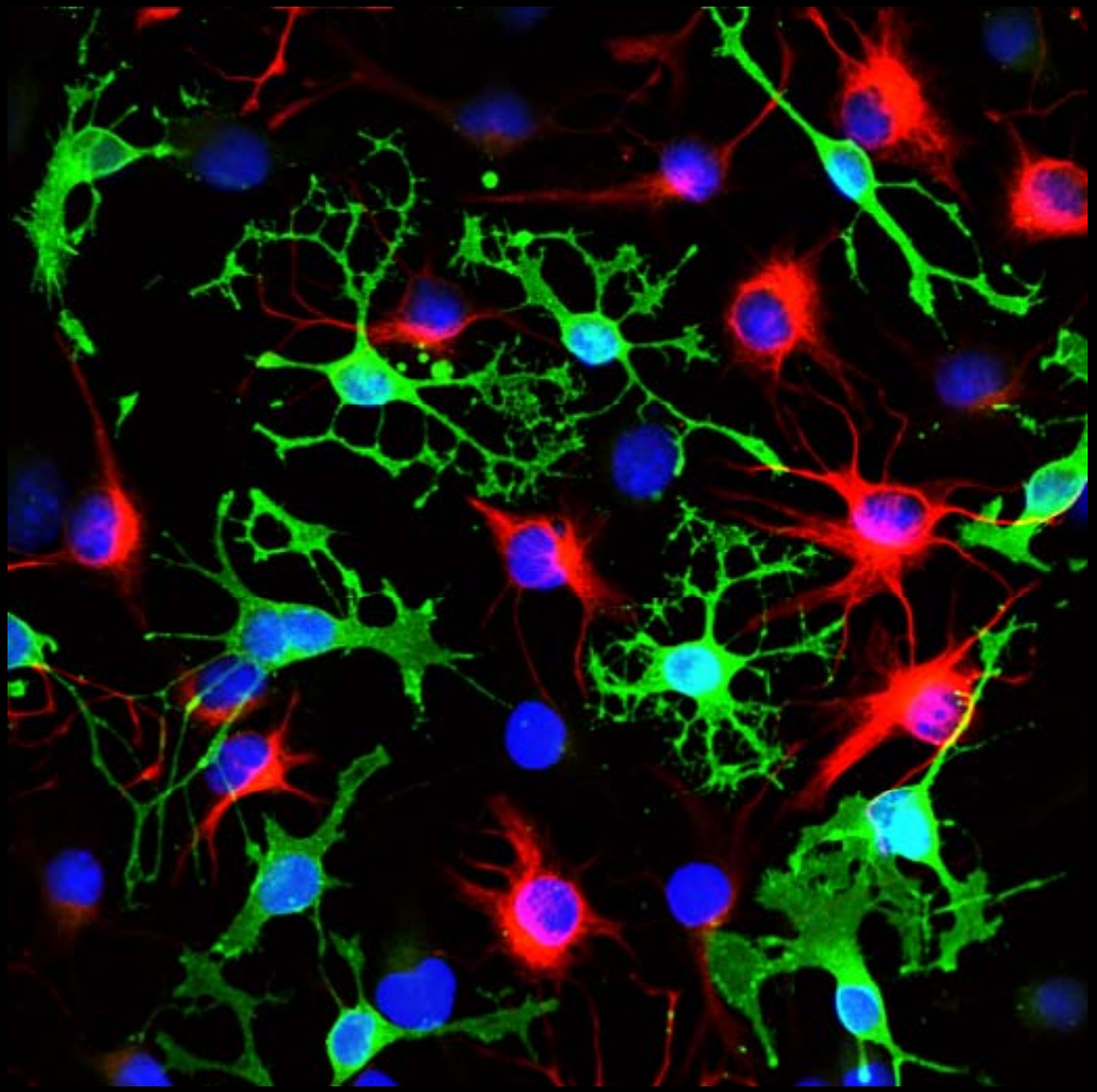
Propagation of Immature Neural Stem Cells



Blue - nucleus

Green - nestin

Differentiation of Stem Cells into Glia

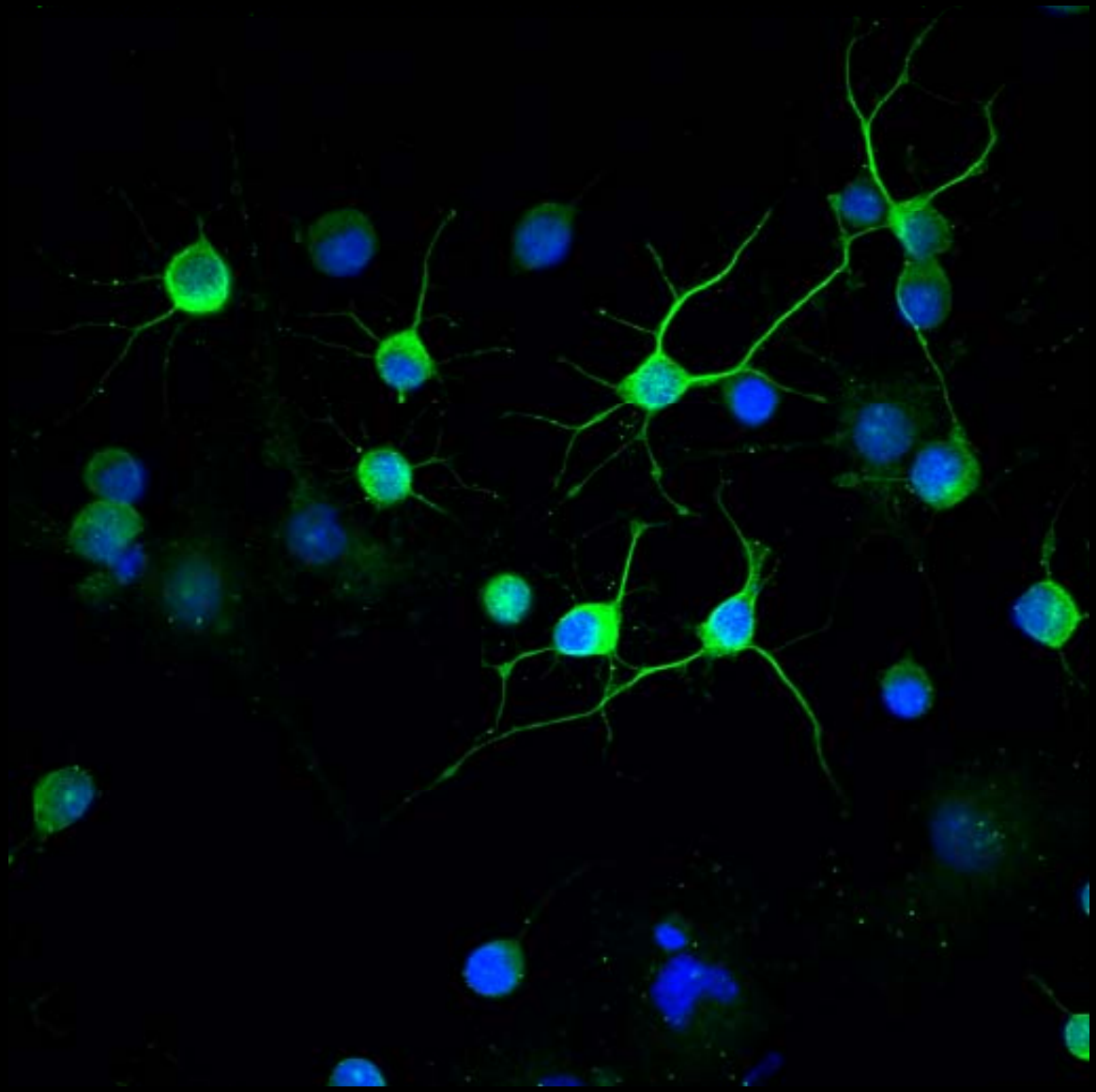


Blue - nucleus

Green - MBP

Red - GFAP

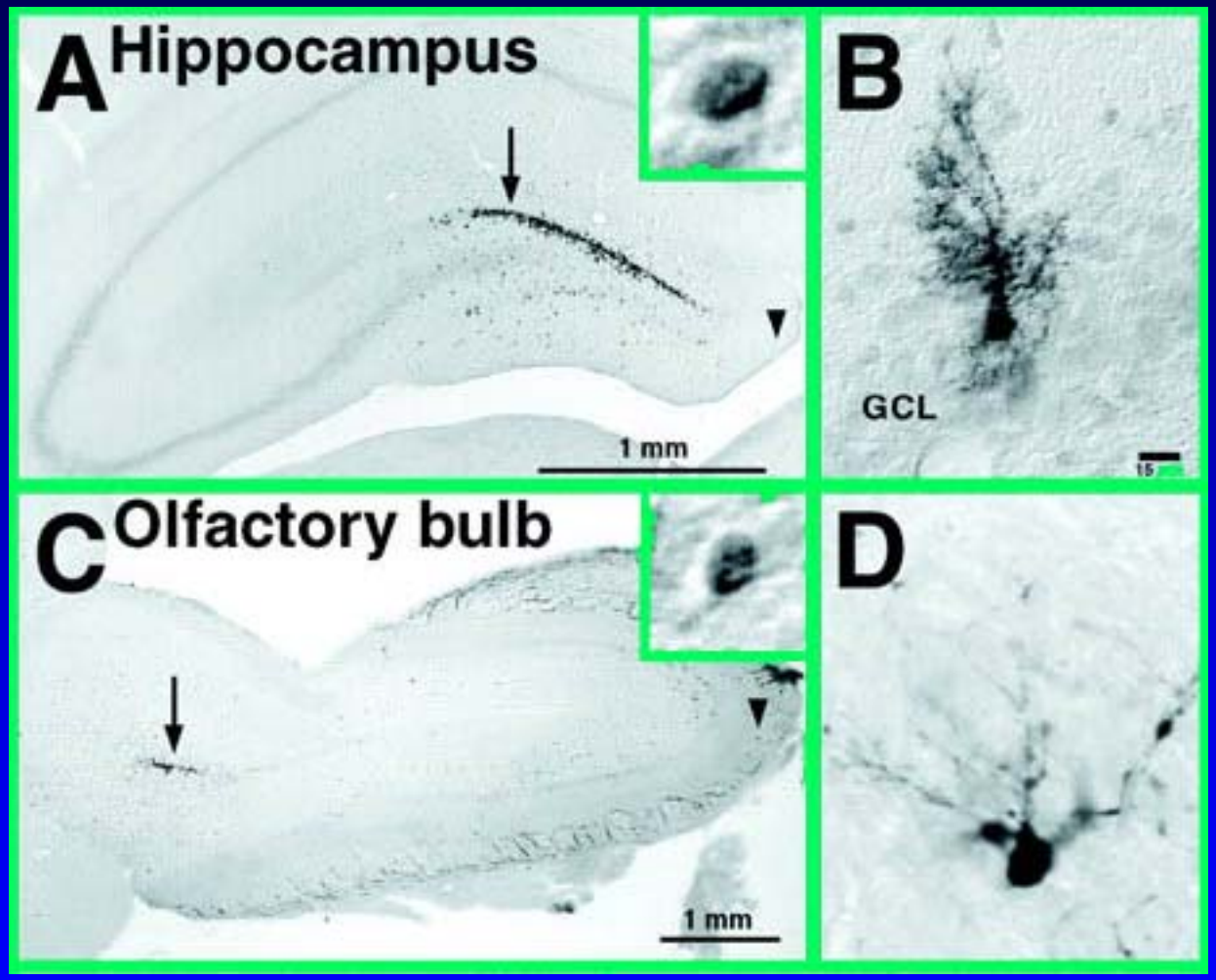
Differentiation into Neurons



Blue - nucleus

Green - NF200

Integration of Implanted Adult Neural Stem Cells

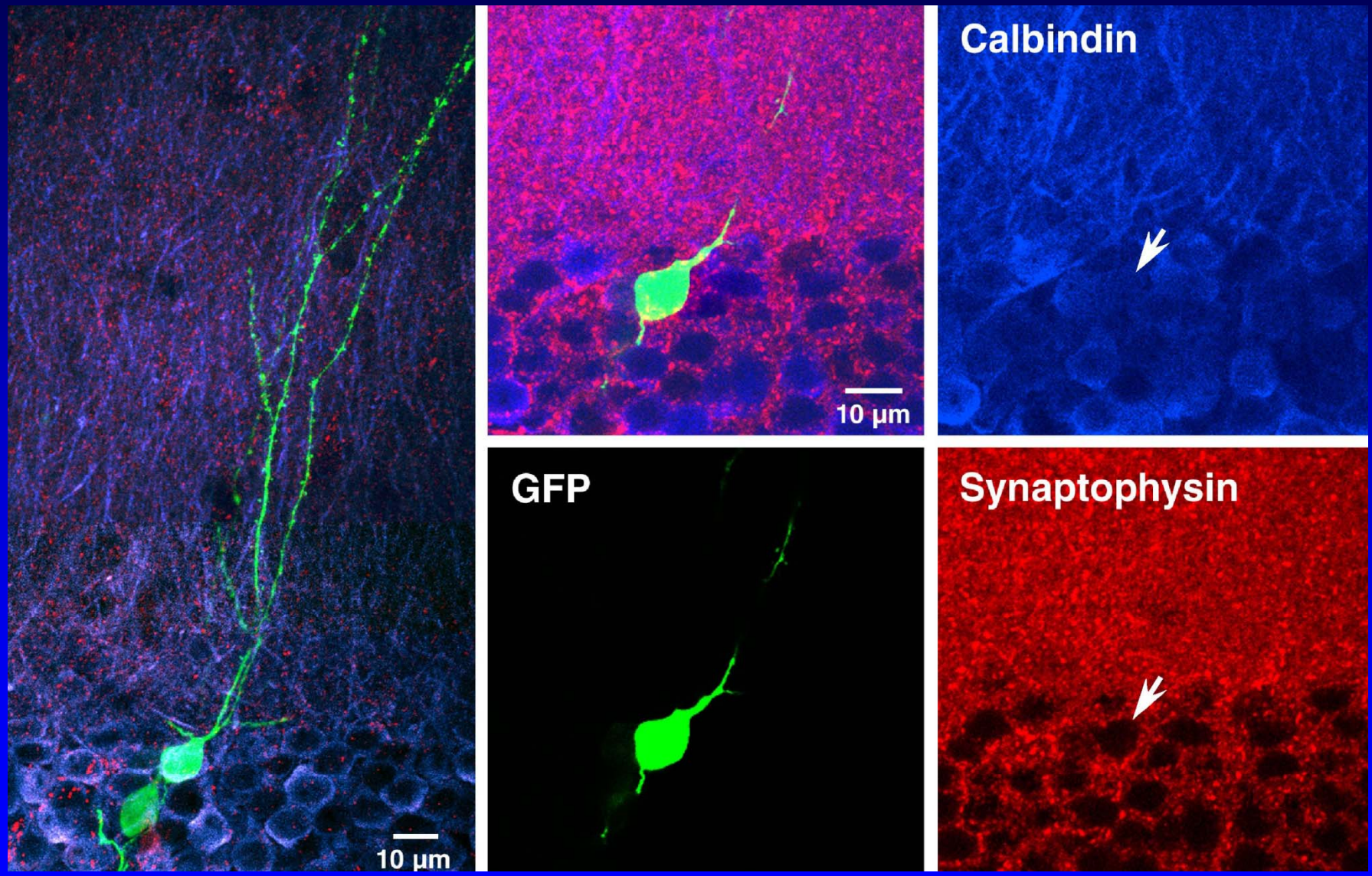


Gage, 1995-1996

Adult Neurogenesis Occurs Across All Mammalian Species

- Mouse
- Rat
- Cat
- Rabbit
- Monkey
- Human

Newborn Hippocampal Neurons are Electrophysiologically Functional



Van Praag and Gage, 2002

Neural Stem Cells Directly Involved in Learning and Memory

nature

Vol 451|21 February 2008|doi:10.1038/nature06562

LETTERS

A role for adult TLX-positive neural stem cells in learning and behaviour

Chun-Li Zhang[1,2], Yuhua Zou[2], Weimin He[2]†, Fred H. Gage[3] & Ronald M. Evans[1,2]

Neurogenesis persists in the adult brain and can be regulated by a plethora of external stimuli, such as learning, memory, exercise, environment and stress[1]. Although newly generated neurons are able to migrate and preferentially incorporate into the neural network[2–5], how these cells are molecularly regulated and whether they are required for any normal brain function are unresolved questions[6]. The adult neural stem cell pool is composed of orphan nuclear receptor TLX-positive cells[7]. Here, using genetic approaches in mice, we demonstrate that TLX (also called NR2E1) regulates adult neural stem cell proliferation in a cell-autonomous manner by controlling a defined genetic network implicated in cell proliferation and growth. Consequently, specific removal of TLX from the adult mouse brain through inducible recombination results in a significant reduction of stem cell proliferation and a marked decrement in spatial learning. In contrast, the resulting suppression of adult neurogenesis does not affect contextual fear conditioning, locomotion or diurnal rhythmic activities, indicating a more selective contribution of newly generated neurons to specific cognitive functions.

population, designated $Tlx^{f/z;CreER}$ NSC, harbours a floxed allele of *Tlx* and a constitutively expressed transgene, $CreER^{TM}$, which encodes a fusion of Cre recombinase and a modified, tamoxifen (TM)-responsive ligand-binding domain of oestrogen receptor[10]. Addition of tamoxifen to the culture medium leads to a temporally controlled robust deletion of the floxed allele of *Tlx* (Fig. 2a). The control population ($Tlx^{f/z}$ NSC) does not contain the $CreER^{TM}$ transgene; thus, treatment with tamoxifen has no effect on *Tlx* mRNA (Fig. 2a).

Using total RNA isolated from these two populations of cells after 36 h or 60 h of treatment with tamoxifen or vehicle, we analysed all genes whose expression was altered by at least 1.39-fold. After exclusion of tamoxifen-induced changes in control cells (36 and 99 genes at 36 h and 60 h, respectively), the number of genes with altered expression in response to deletion of *Tlx* was found to be 432 genes at 36 h and 607 genes at 60 h (Fig. 2b). Among these genes, 53.9% and 51.7% are upregulated at 36 h and 60 h, respectively (Supplementary data 1–3). Further analysis revealed that 206 genes had altered expression levels at both 36 h and 60 h after tamoxifen-induced

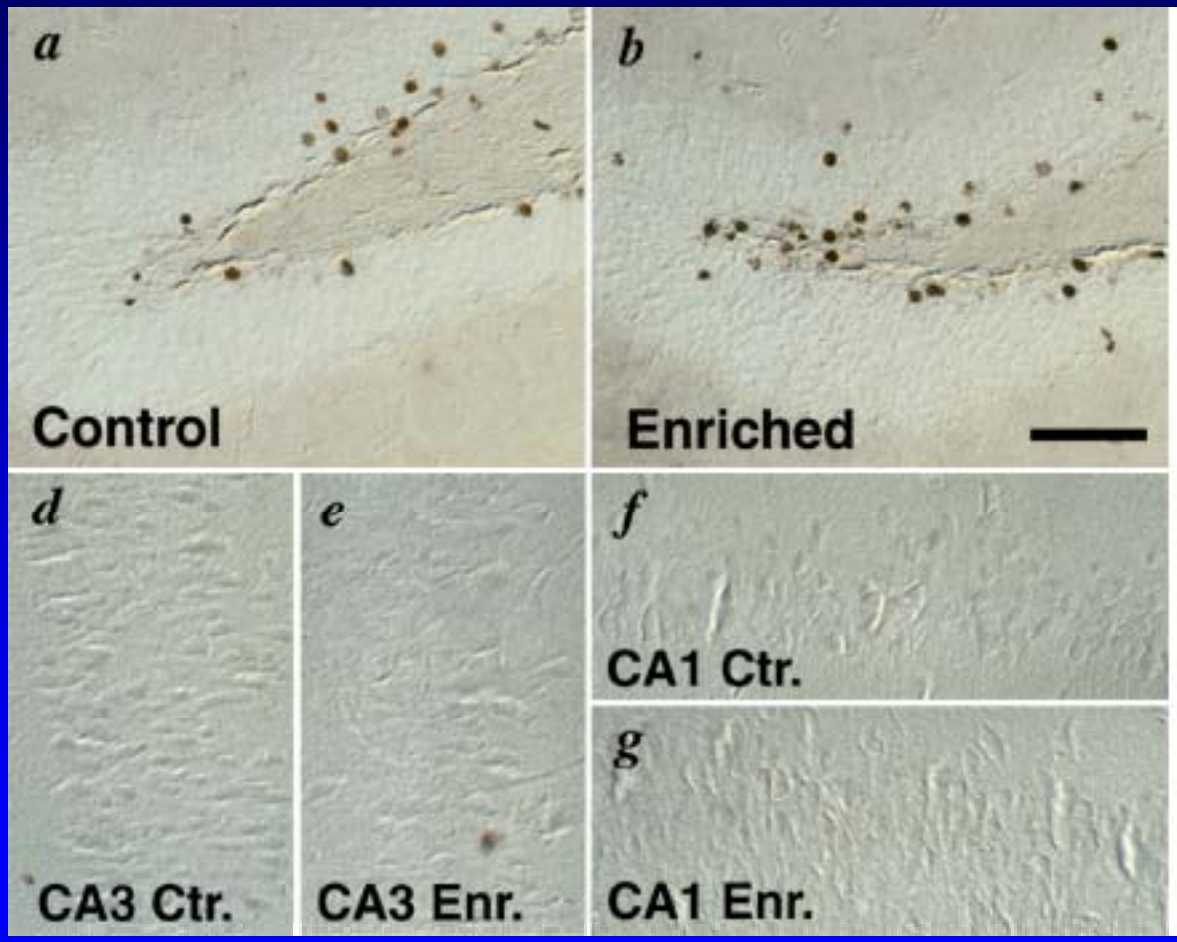
Regulation of Neurogenesis

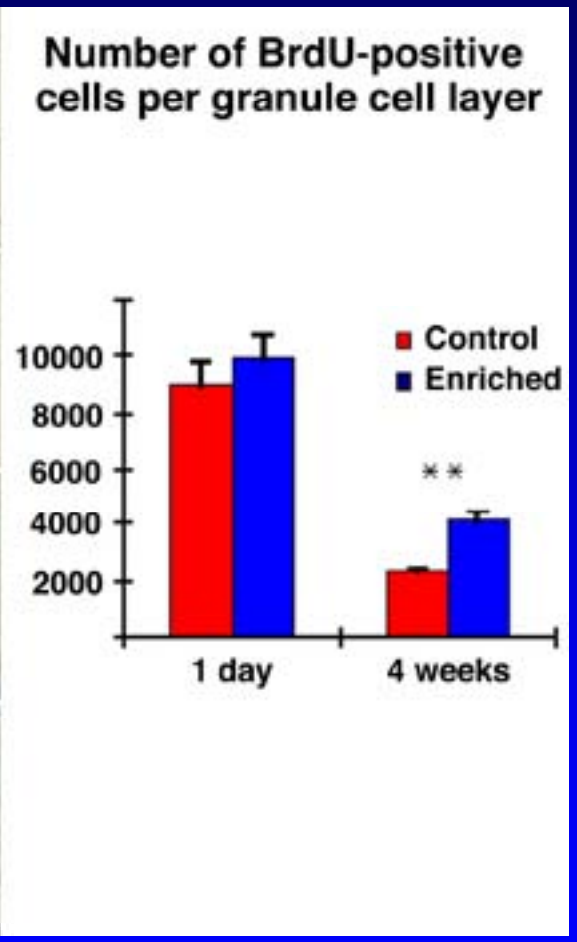
- Enriched environment
- Exercise
- Stroke
- Epilepsy
- Diet
- Learning
- Stress
- Aging
- Depression
- Hormones
- Neurotransmitters
- Growth factors

Environmental Enrichment Enhances Hippocampal Neurogenesis and Learning

Kempermann et al., 1997

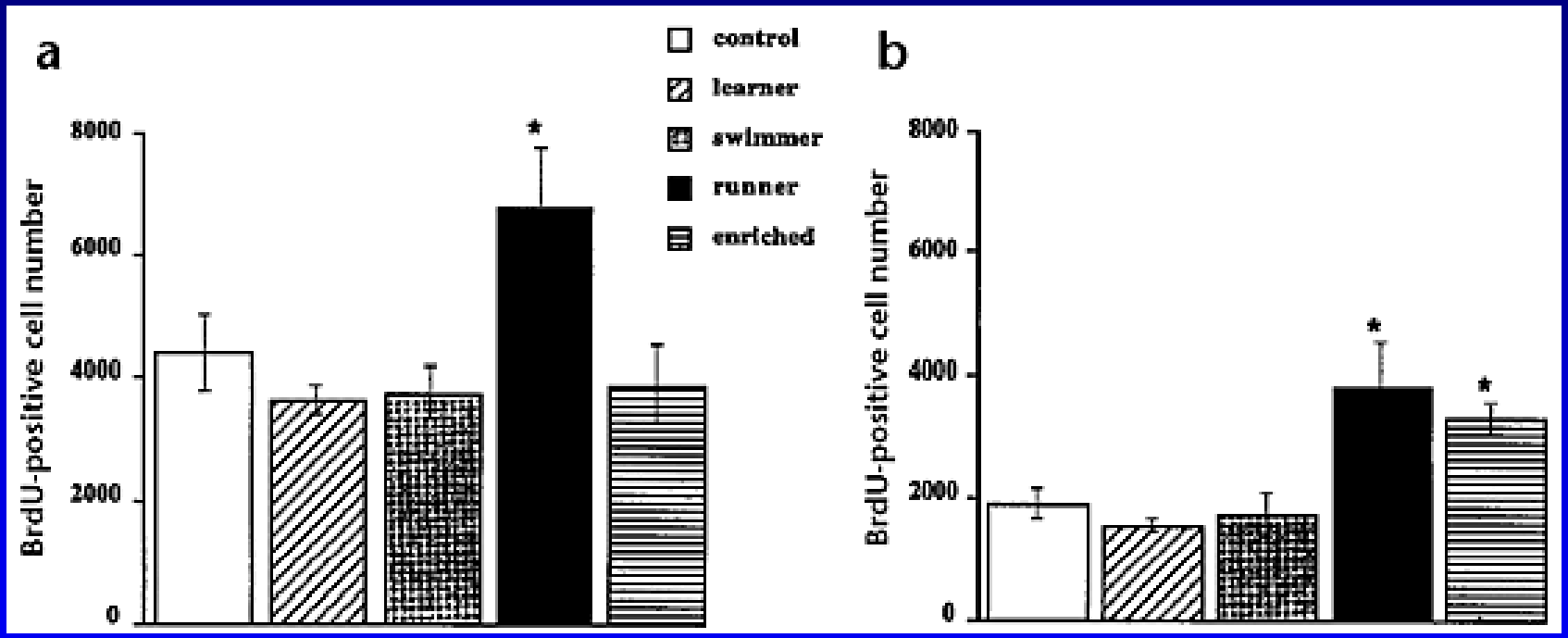
Environmental Enrichment Enhances Hippocampal Neurogenesis and Learning



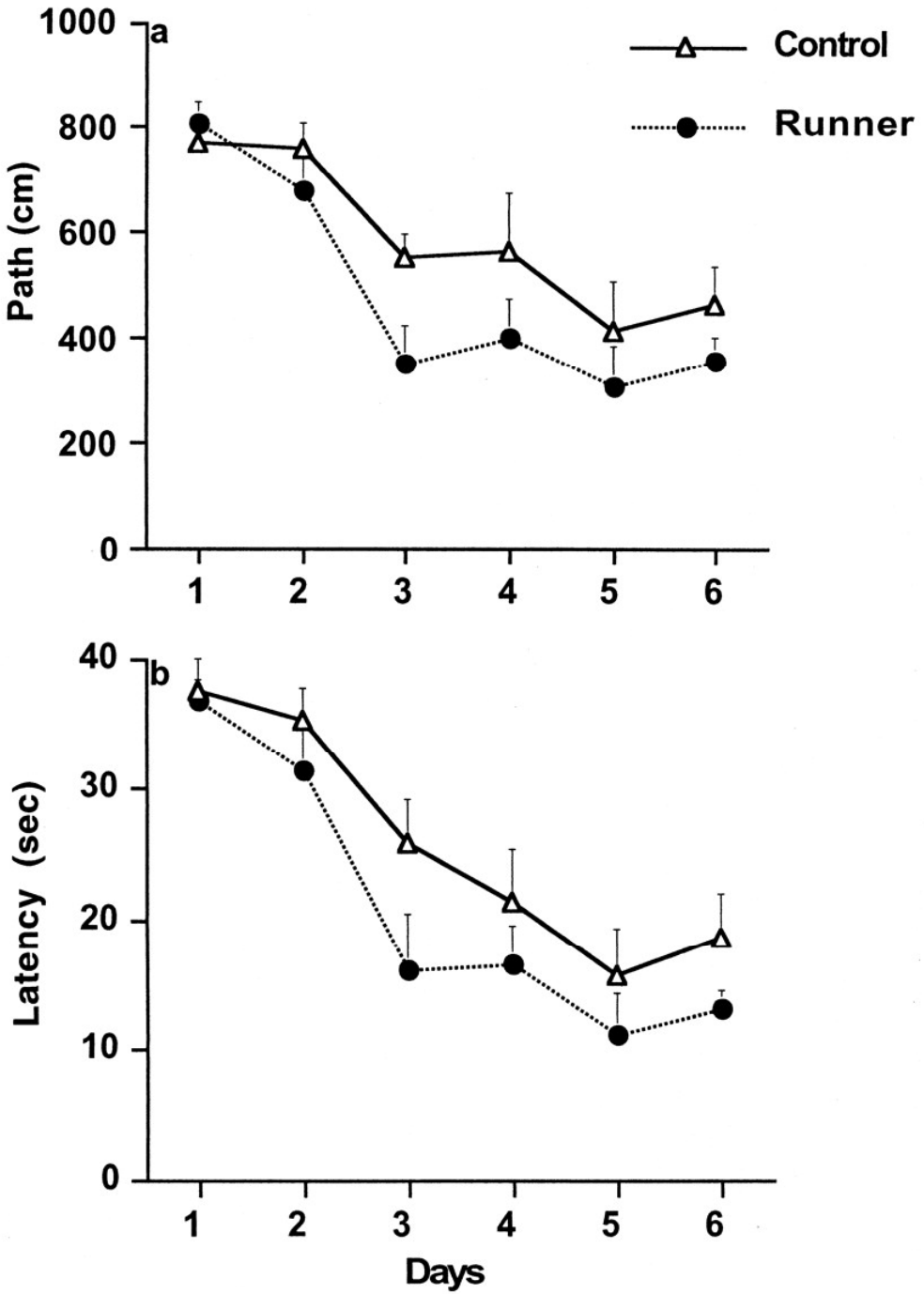


Kempermann et al., 1997

“Dissection” of Environmental Enrichment



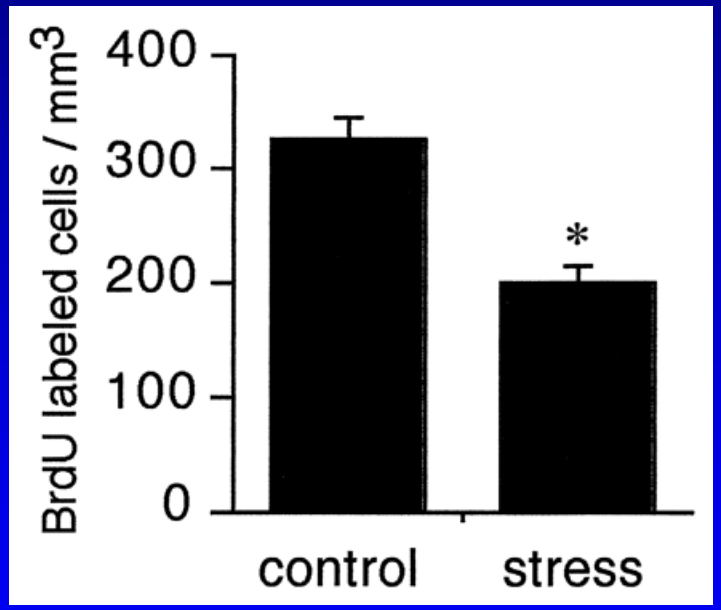
Van Praag et al., 1999



Exercise Enhances Neurogenesis and Learning

Van Praag., 1999

Stress Inhibits Neurogenesis

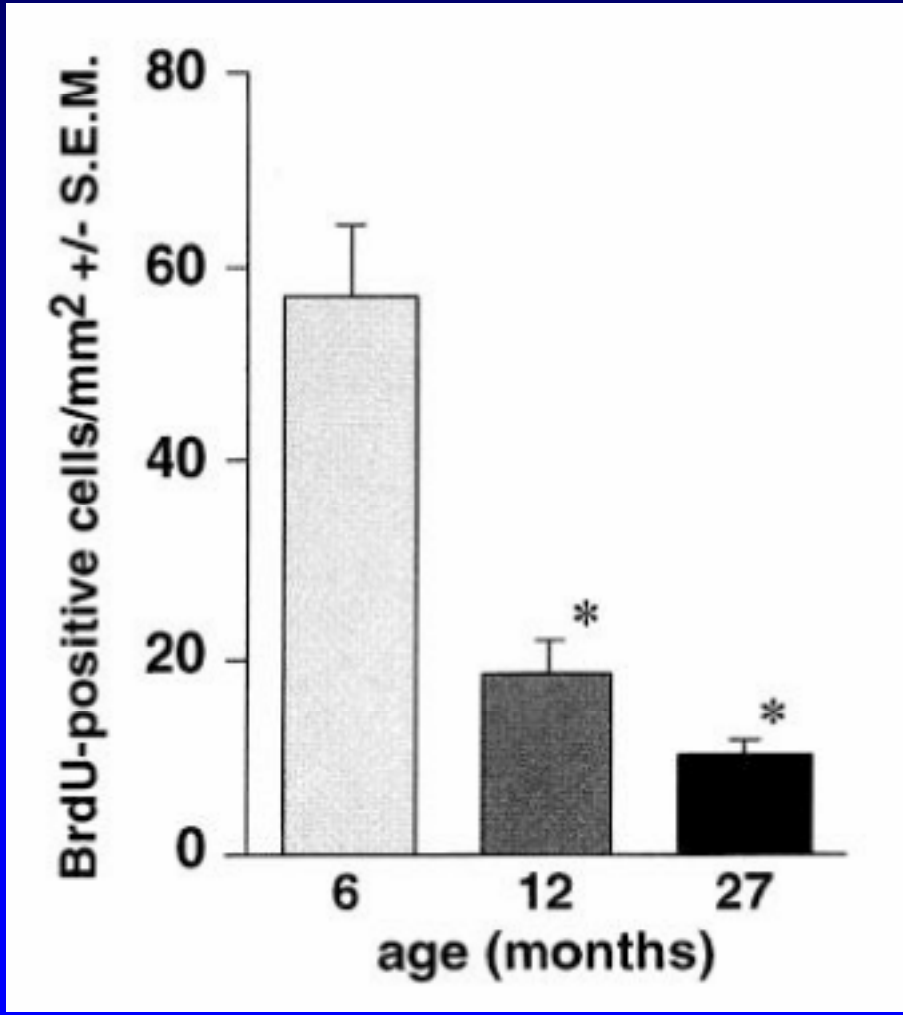


Various inducers of stress robustly reduced neural progenitor proliferation in the rodent and primate brain.

Mediated by glucocorticoids.

Gould et al., 1997, 1998

Hippocampal Neurogenesis Decreases with Age

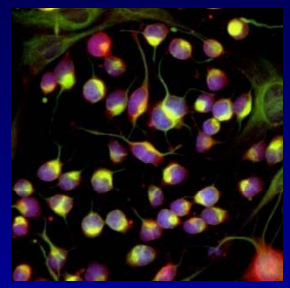


Aged rats have <10% the rate of new neuron degeneration as their young counterparts.

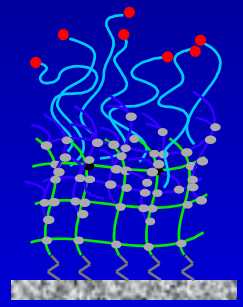
Kuhn and Gage, 1996

Outline

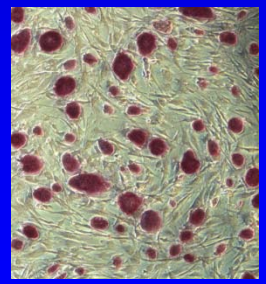
- Neural stem cells



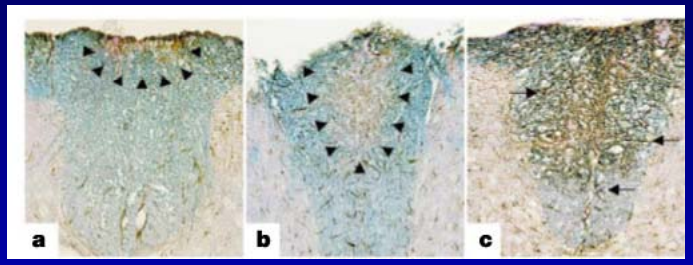
- **Understanding and engineering the stem cell microenvironment**



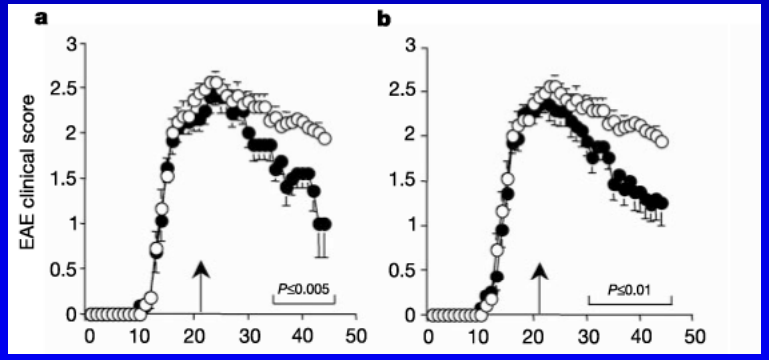
- Recent developments in the field



Injected Adult NSCs are Therapeutic in Multiple Sclerosis Model



Reduced glial scarring

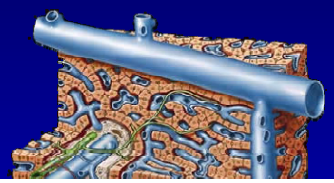


Enhanced motor performance

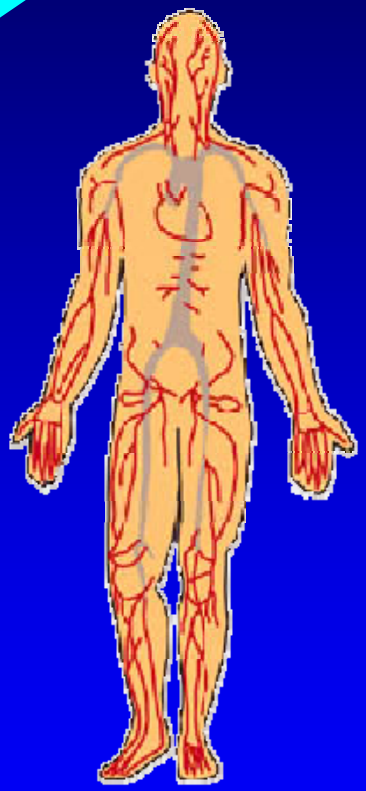
Vescovi et al., 2004

Cell Replacement Therapy: The Vision

Engineer Tissue



Engraft



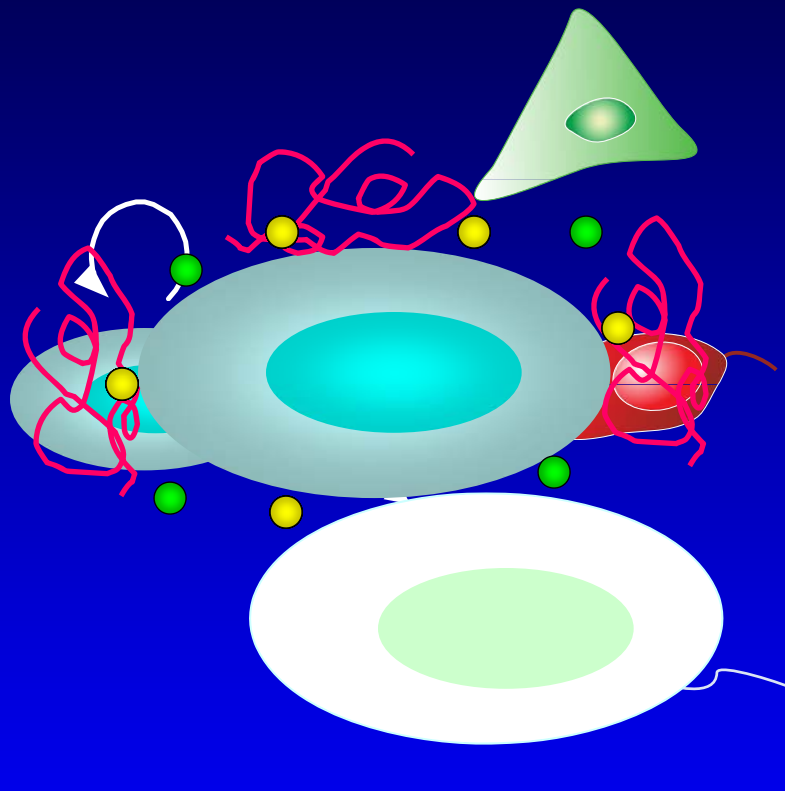
Challenges:

- Viability
- Functional integration
- Biocompatibility, safety

Expand

Isolate

Stem Cell Microenvironment



Growth Factors & Morphogens

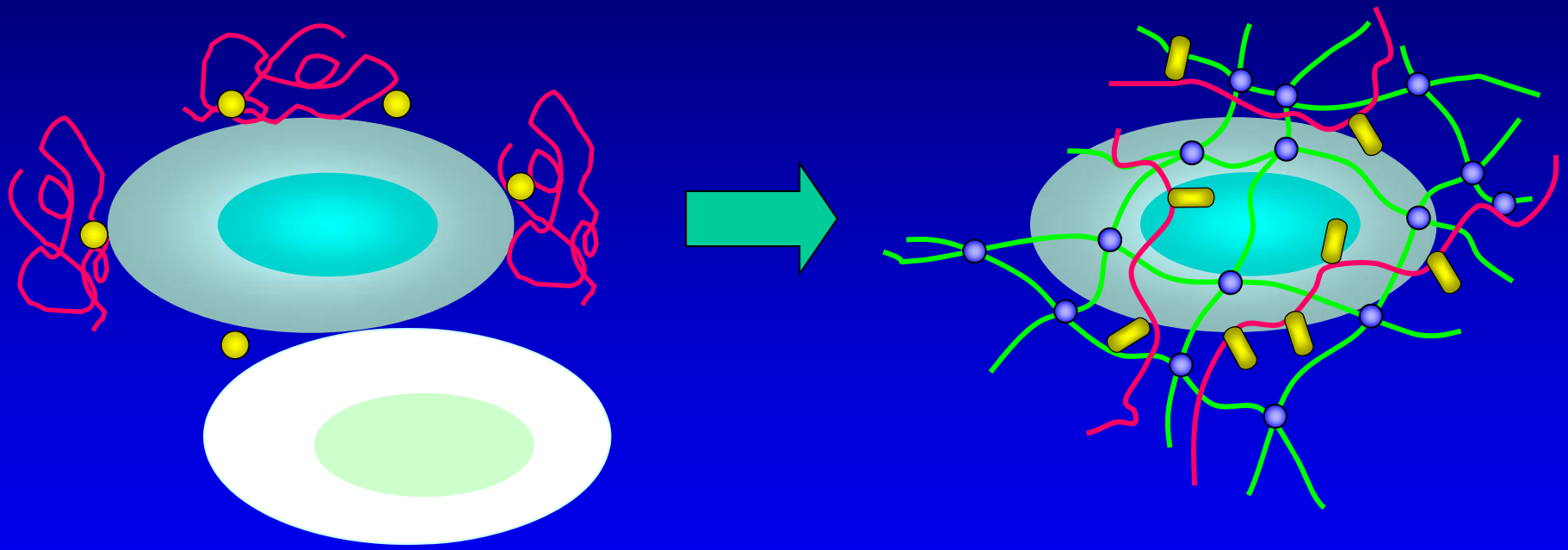
Small Molecules

Extracellular Matrix

Other Cells

Complex dynamic and reciprocal interact to regulate stem cell function

Understand, then Engineer Stem Cell Microenvironments to Control Their Function

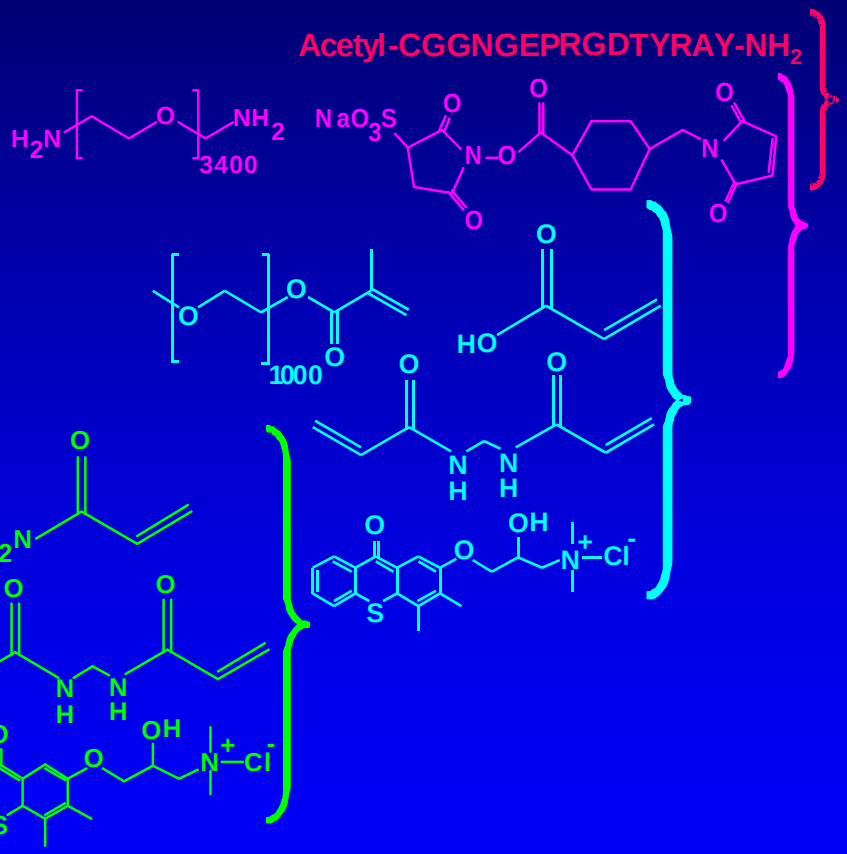


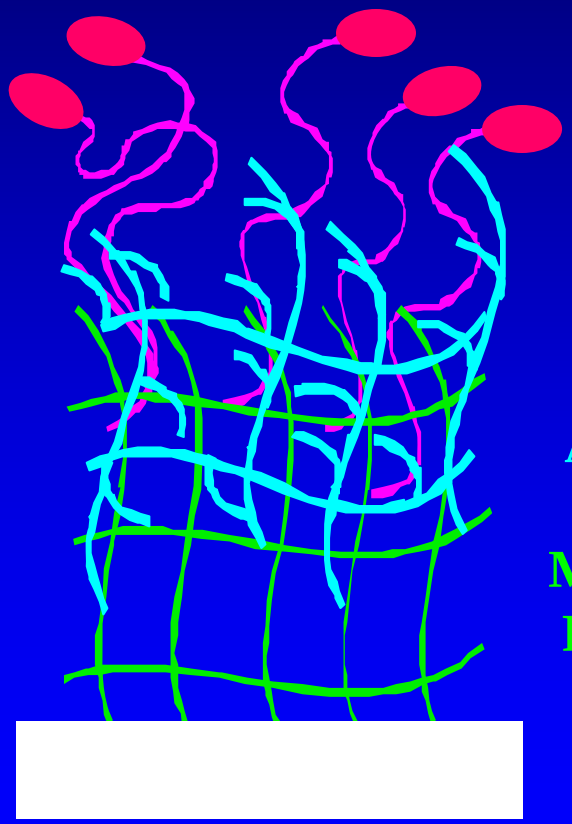
Engineer microenvironments that present these cues to aid in cell expansion, differentiation, assembly, and implantation

Goal: Engineer Synthetic Microenvironments for Cell Expansion, Differentiation, and Implantation

- Maintain precise control over cell function
- Distill complexity of the environment
- Biocompatible
- Safe, reproducible, scaleable
- Animal/human protein free

Synthesis of a Peptide-Grafted Synthetic Microenvironment





Signaling Ligands

Protein Adsorption

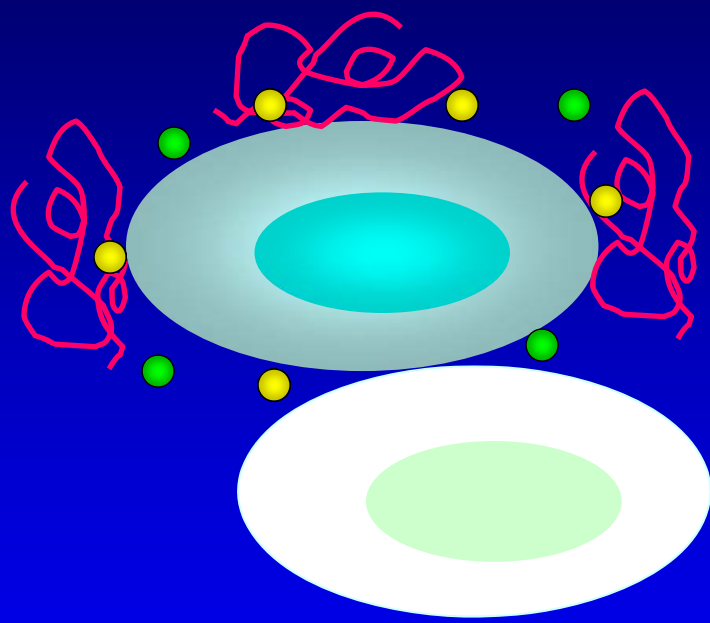
Mechanical Properties

Bearinger, J. P., *et al., J. Biomat. Sci. Polym. Ed.*, 9(7), 629, 1998

Stem Cell Microenvironment

Growth Factors & Morphogens: Immobilization

Small Molecules

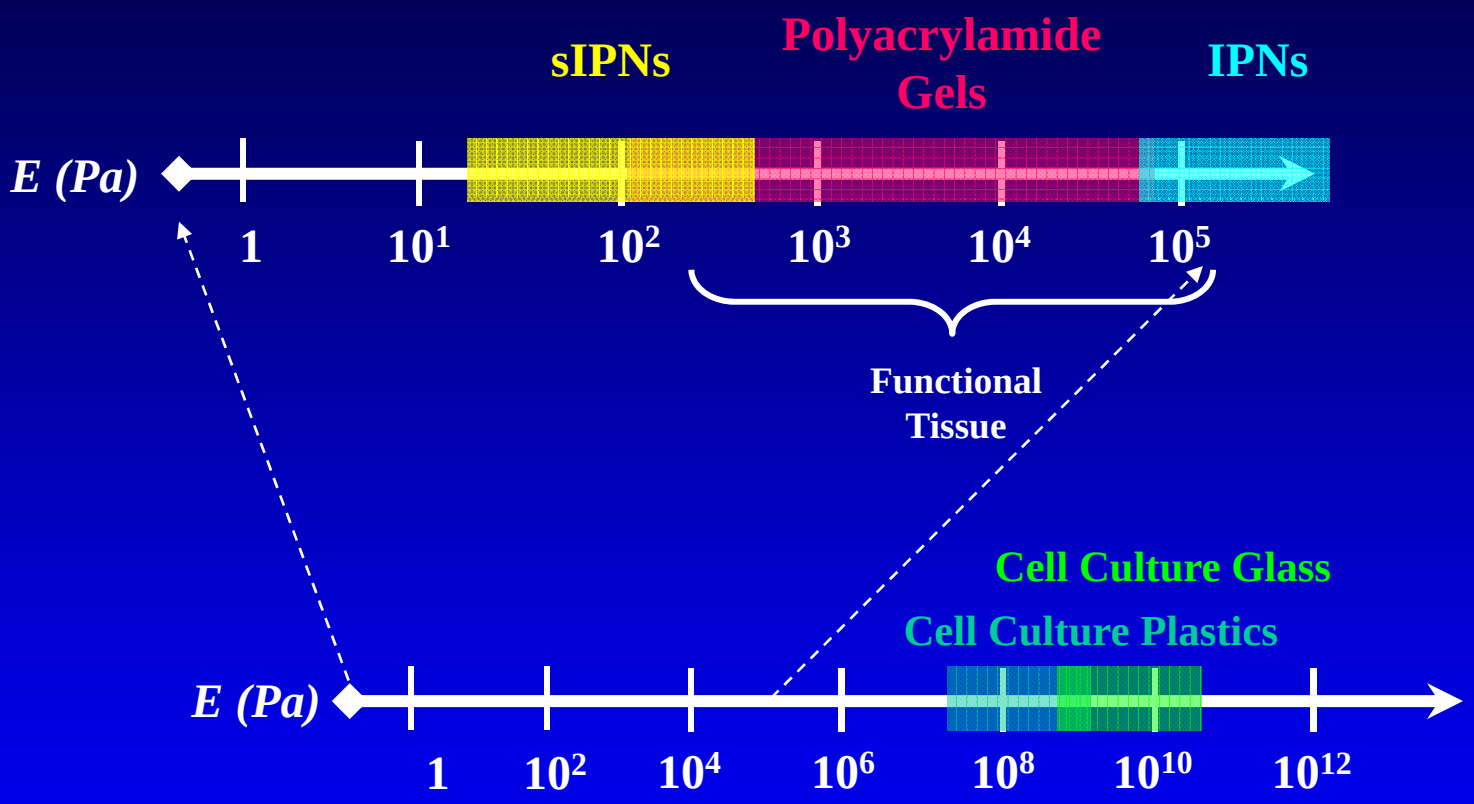


Extracellular Matrix: Multiple Motifs, Solid Phase

Other Cells: Spatial Organization of Ligands

Mechanics is increasingly recognized as important in regulating stem cell behavior

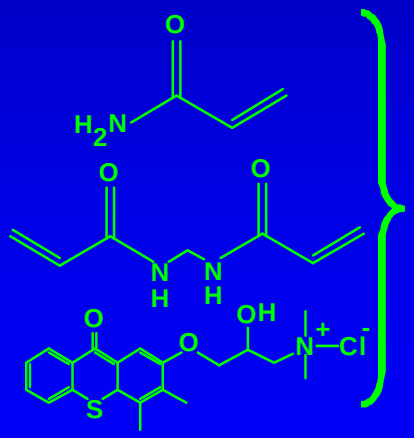
Range of Cell Substrate Elasticity



Does *E* affect cell self-renewal and/or differentiation?

Sheu MT, *et al.* 2001. *Biomaterials* 22(13):1713 & Engler/Discher, UPenn

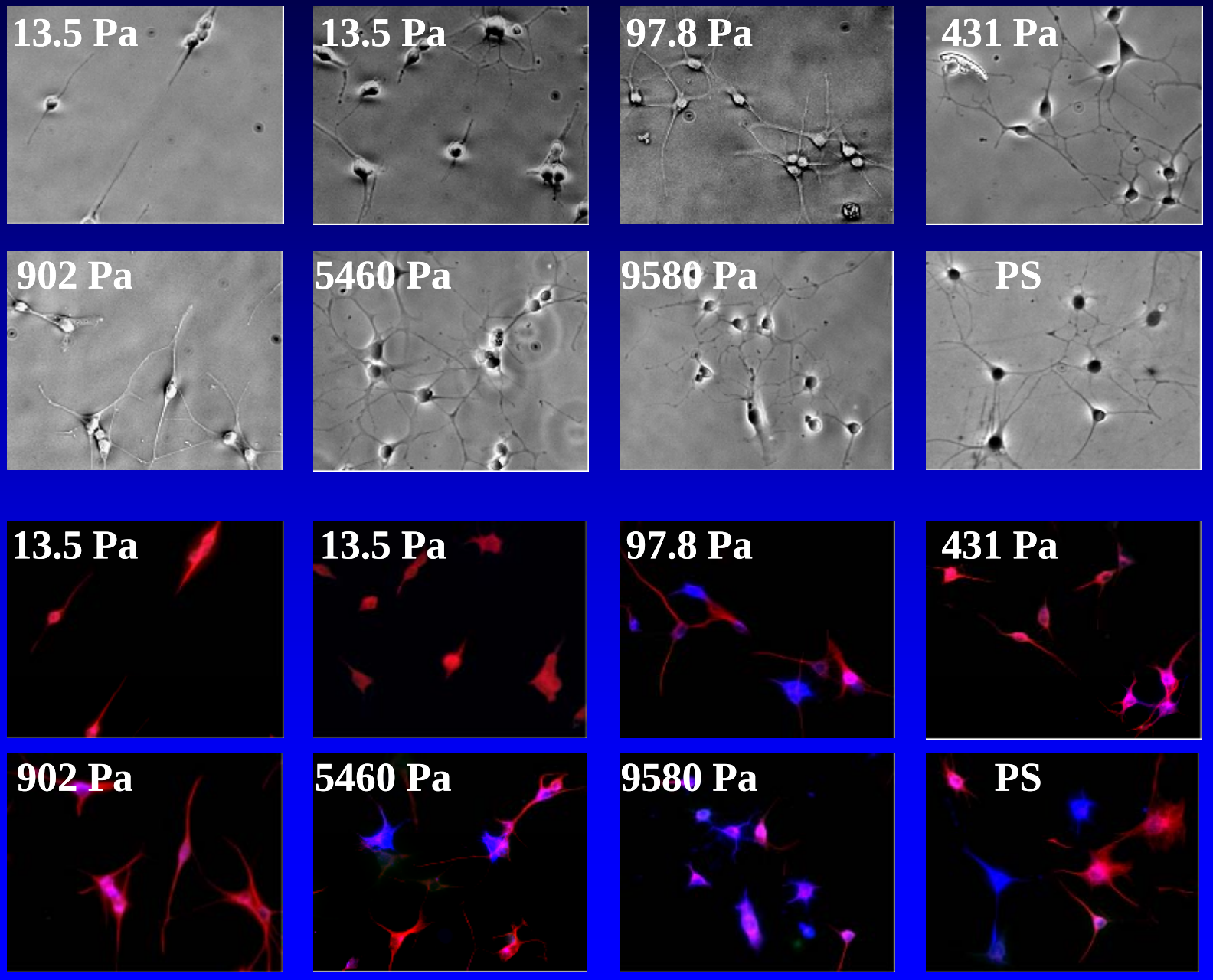
Synthesis of a Peptide-Grafted Hydrogel Microenvironment



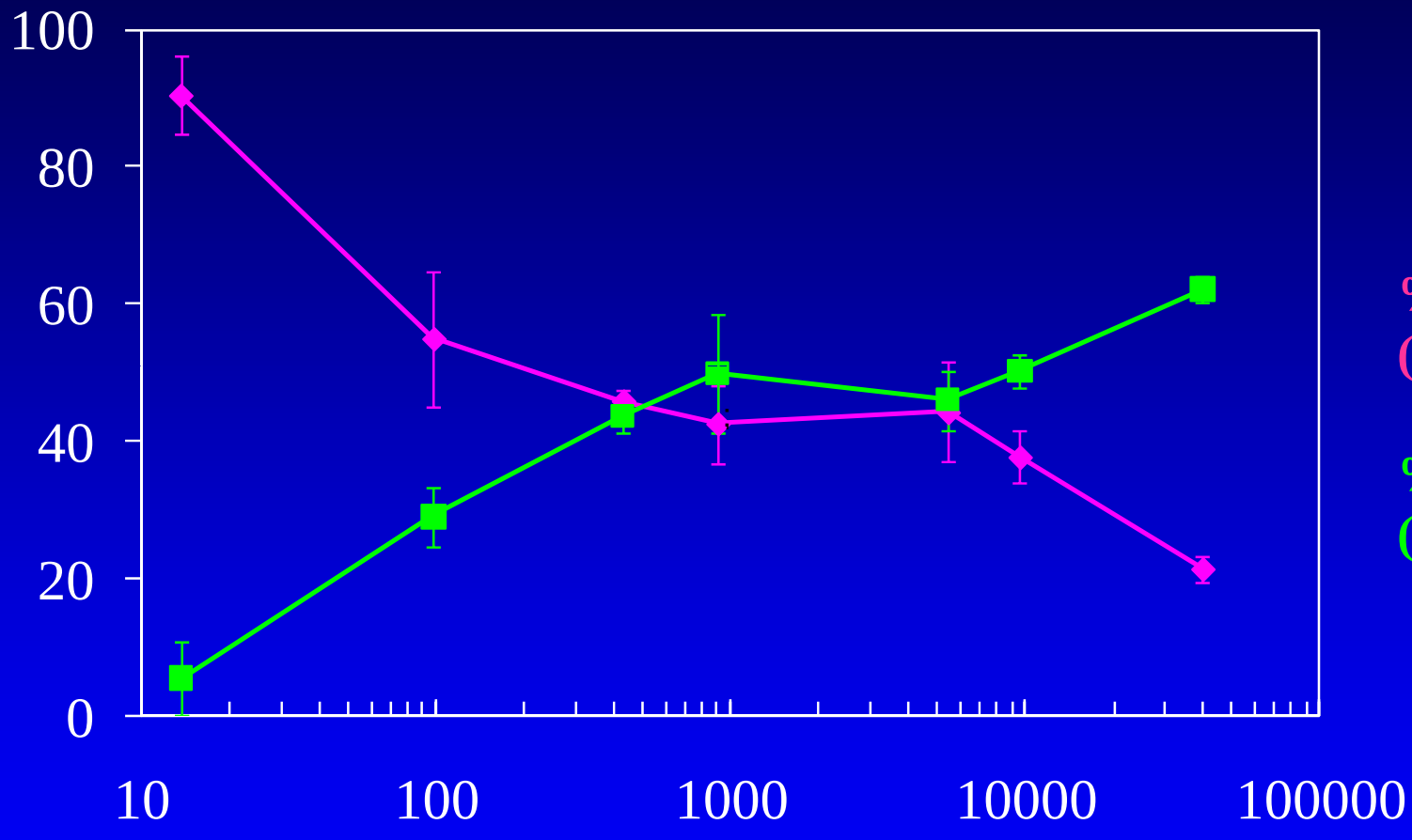
Mechanical Properties

Bearinger, J. P., *et al., J. Biomat. Sci. Polym. Ed.*, 9(7), 629, 1998

Nonspecific Differentiation Conditions

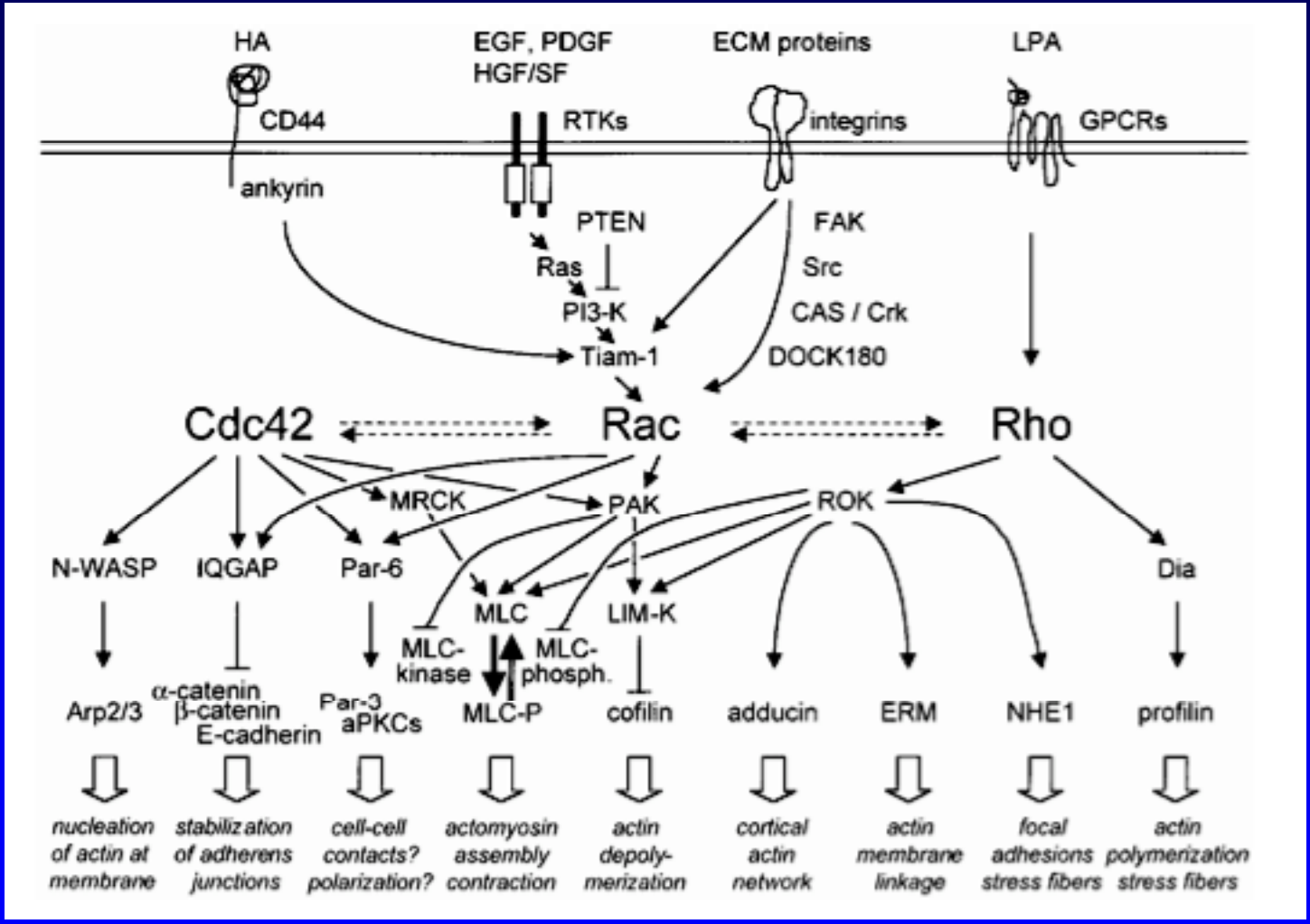


Mixed Differentiation Conditions



Saha et al., Biophys. J. *(2008)*

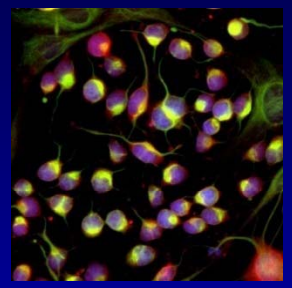
Mechanotransduction



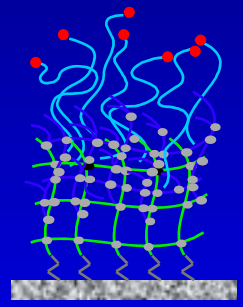
Ingber J., Cell Sci. *(2003)*

Outline

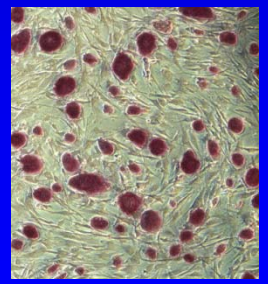
- Neural stem cells



- Understanding and engineering the stem cell microenvironment



- **Recent developments in the field**

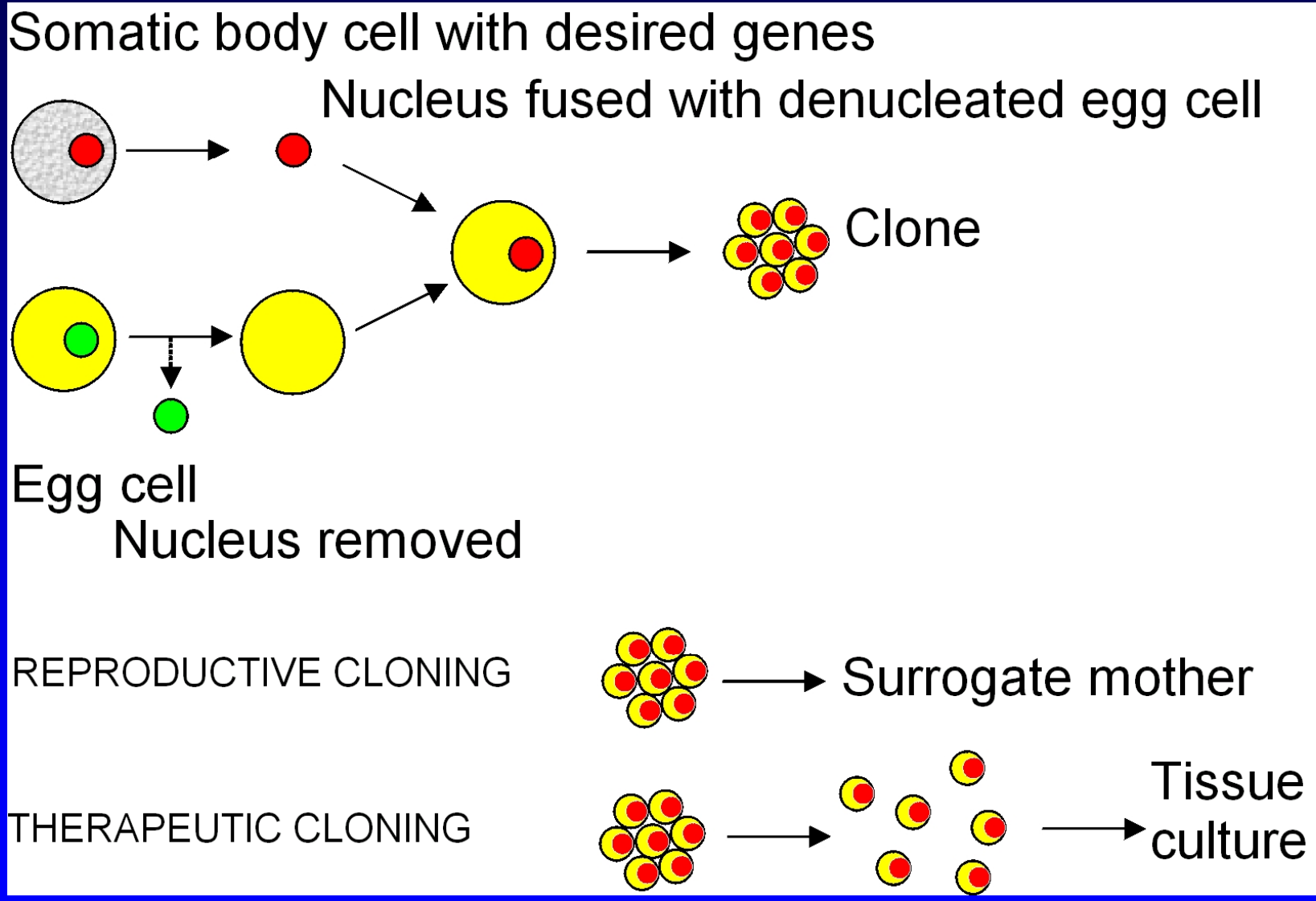


What are Sources of Pluripotent Stem Cells?

- Embryonic stem cells
  derived from embryos

- Somatic cell nuclear transfer

What is cloning (SCNT)?

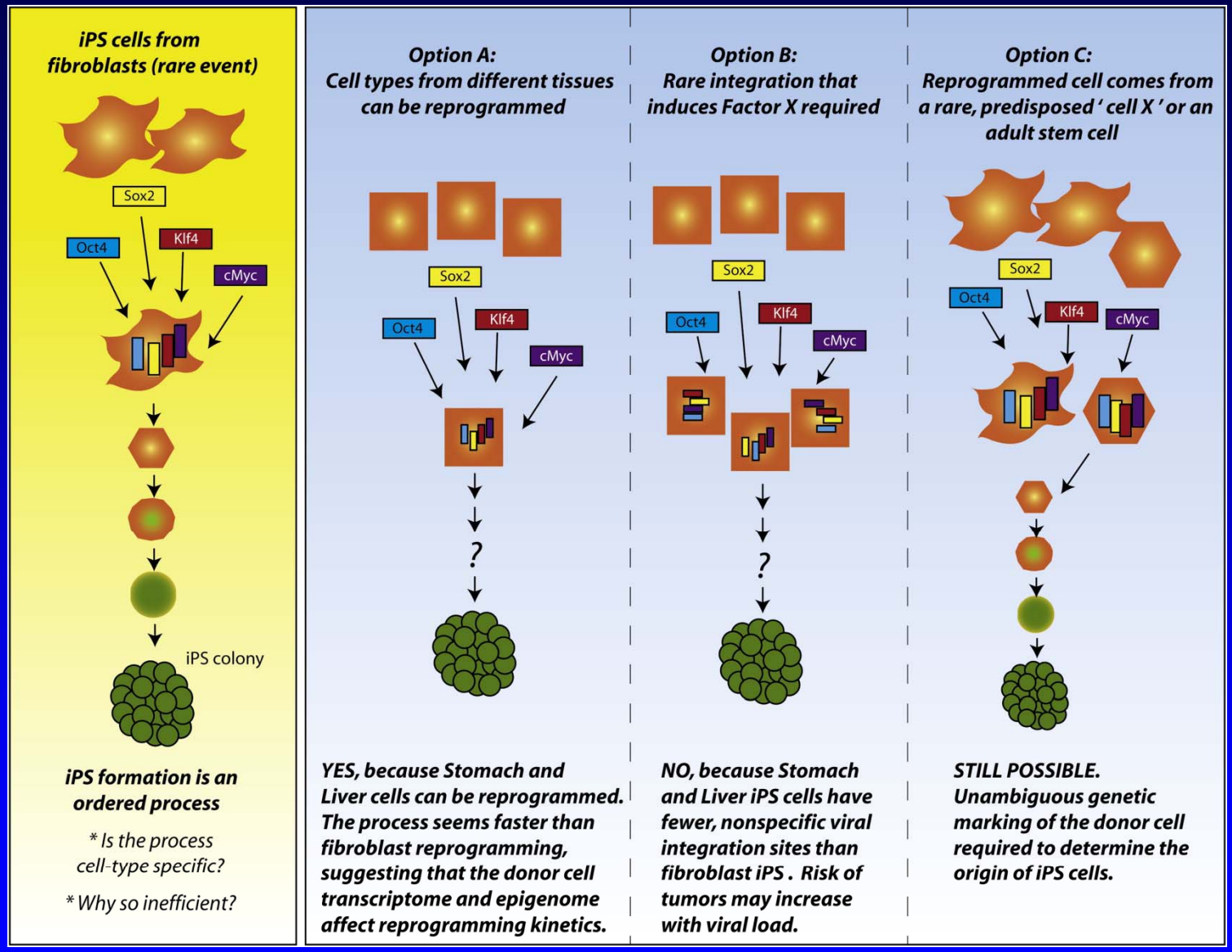


Wikipedia.org

What are Sources of Pluripotent Stem Cells?

- Embryonic stem cells
  derived from embryos
- Somatic cell nuclear transfer
  never successful for human cells
- Induced pluripotent (reprogrammed) cells
  highly promising, but early stage research

Cellular Reprogramming



Sridharan and Plath, 2008

Applications of Reprogramming

- The generation of personalized stem cells that may overcome issues with immune rejection.
- The development of new models to study human disease.
- High throughput pharmacology/toxicology screening.

Summary

- Must better understand and engineer microenvironmental signals, both biochemical and mechanical, that control stem cell function.
- Synthetic matrices can emulate numerous aspects of the cellular microenvironment, including ECM motifs, stiffness, and nanostructured signals.
- Resulting synthetic hydrogel supports neural stem cell self-renewal and differentiation & short term human embryonic stem cell self-renewal.
- System is chemically-defined, biocompatible, and scaleable.