Reprogramming of fibroblasts to neurons can be achieved by:

A. Transplanting fibroblasts in the brain

B. Expressing a combination of transcription factor in fibroblasts

C. Transplanting fibroblasts in a blastocyst

D. Exposing fibroblasts to soluble neural growth factors

Is (are) true concerning plasticity:

A. High plasticity = potential to differentiate into many different cell types

B. Stem cells typically become more plastic during aging

C. Highly plastic cells are more difficult to differentiate into another cell type

D. Plastic cells typically contain large domains of heterochromatin

Neural stem cells

David Suter – slides (most) from Marlen Knobloch, PhD
Institute of Bioengineering, EPFL
david.suter@epfl.ch

What you will learn during this lecture:

Neural stem cells during brain development

- what are neural stem cells?
- how diverse are neural stem cells?

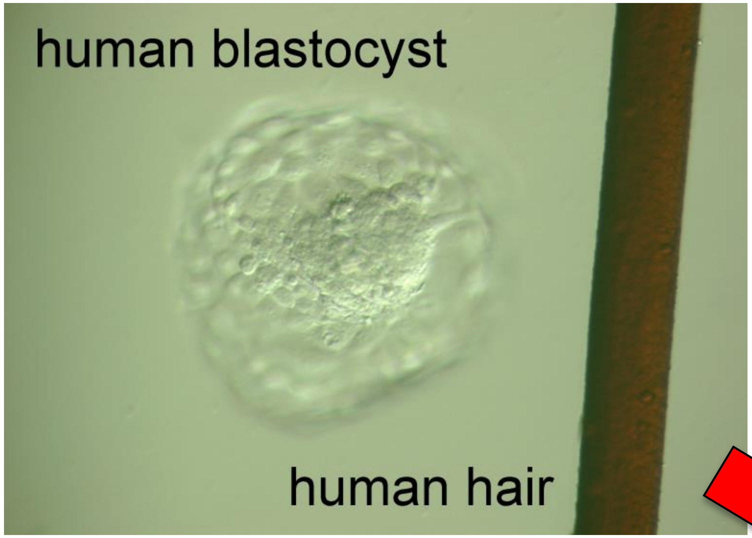
Neural stem cells in the adult brain

- how can adult neural stem cells be studied?
- what regulates adult neural stem cells?
- what is the functional contribution of adult neural stem cells?

Neural stem cells and therapy

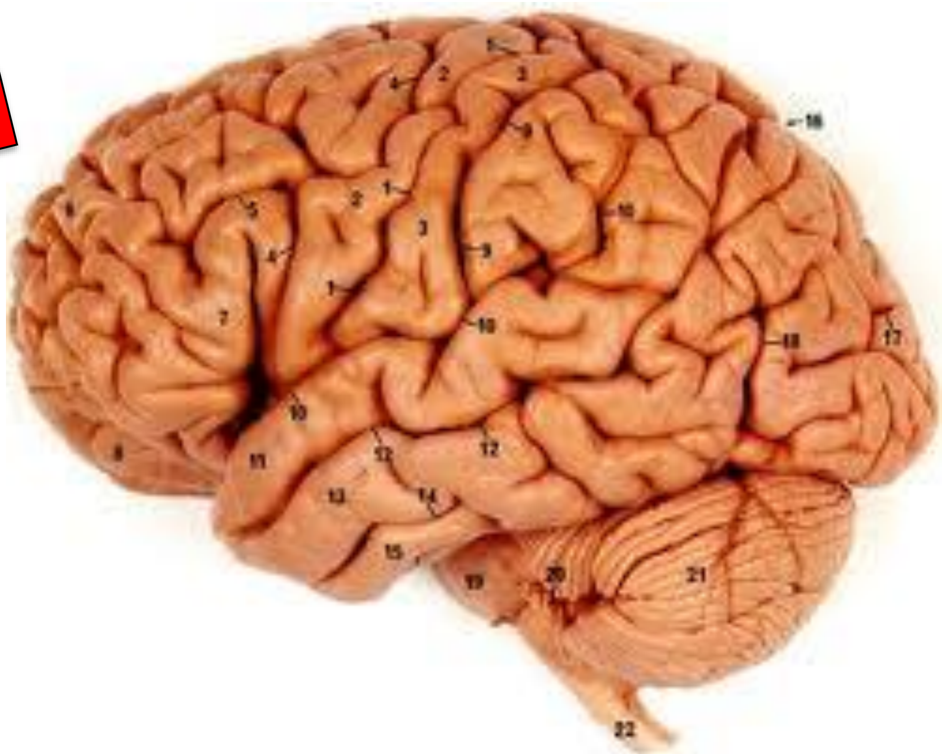
- hope for degenerative diseases?

Brain Development



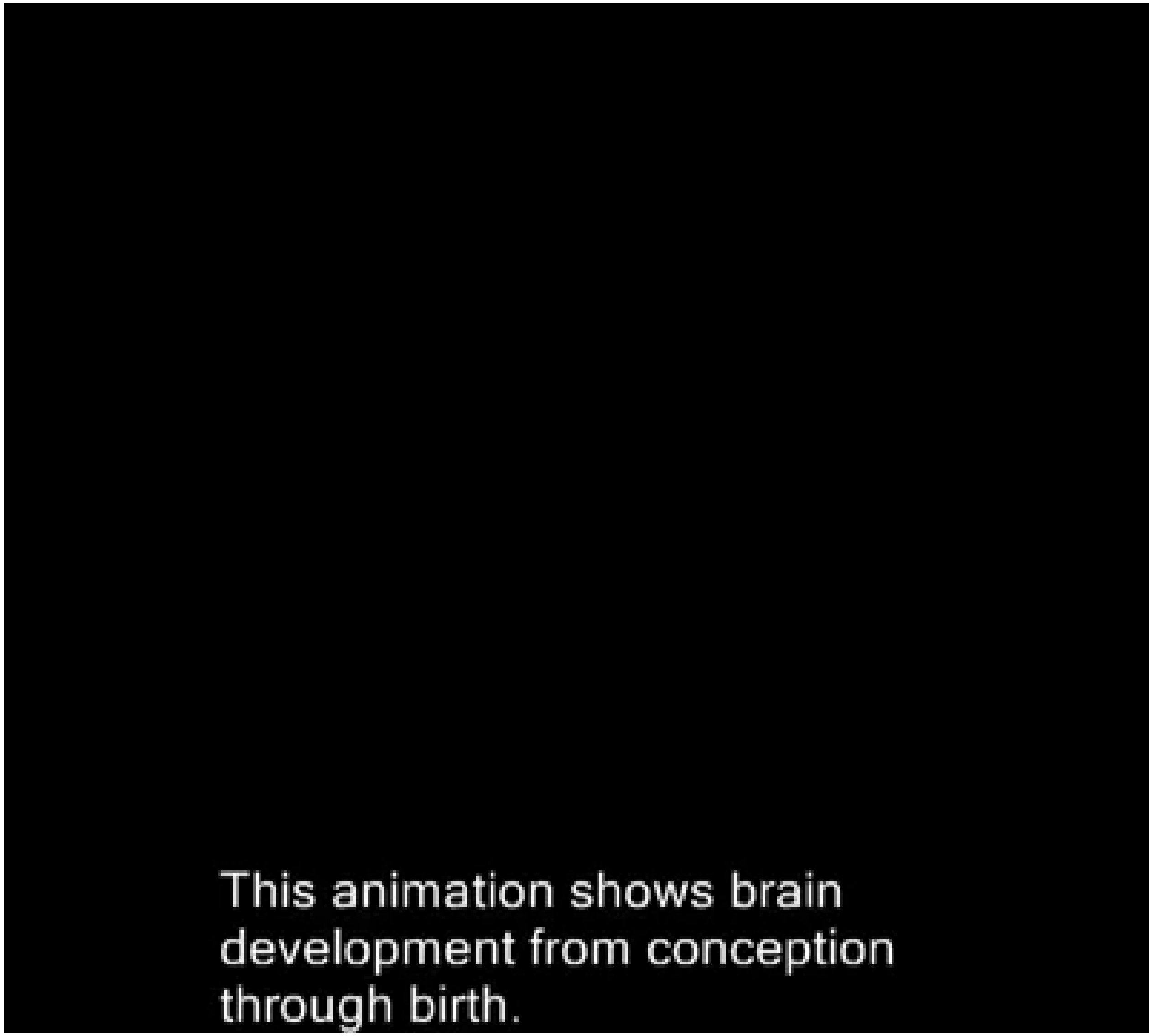
eurostemcell.org

?



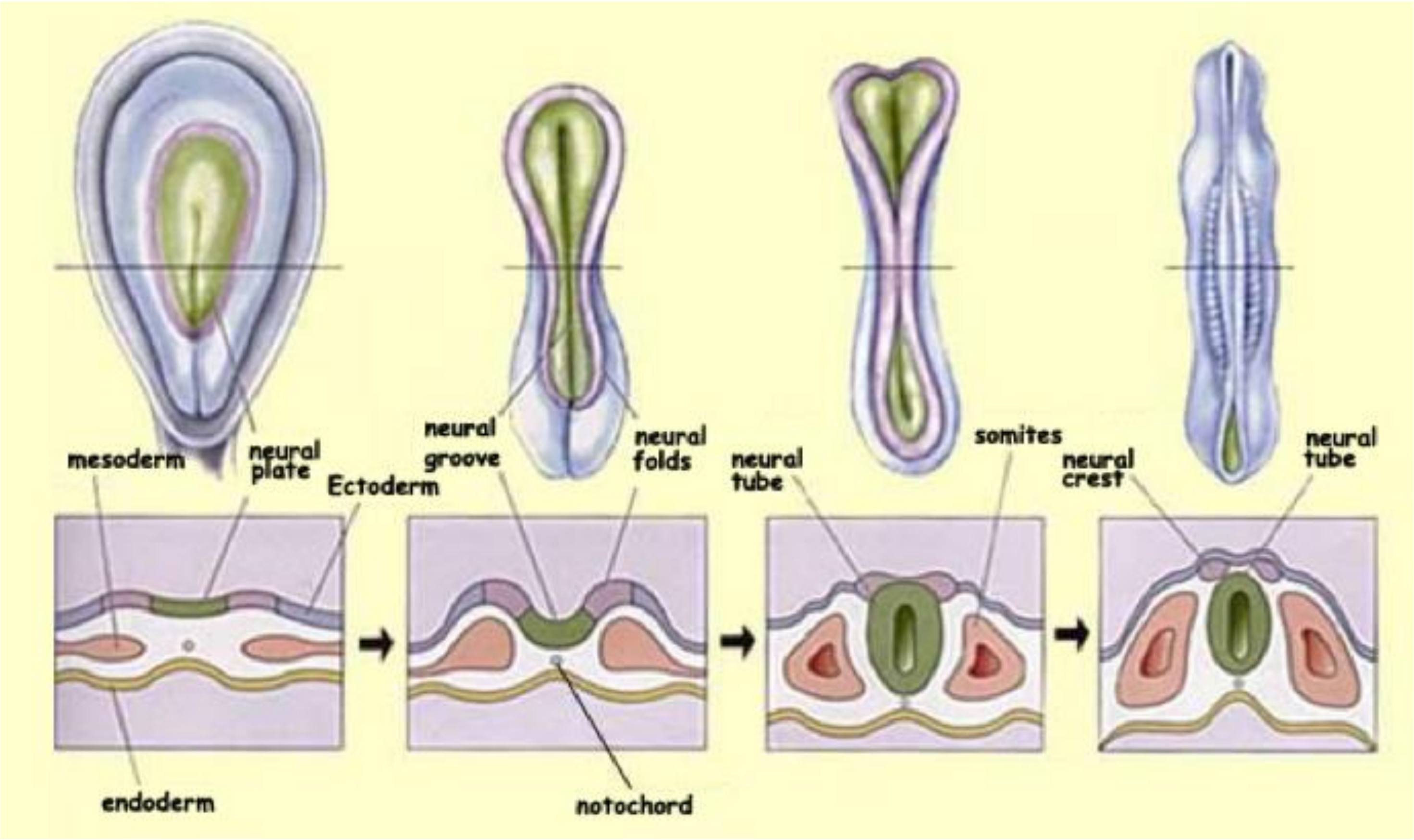
~1.5kg, ~1200cm^3

Brain Development



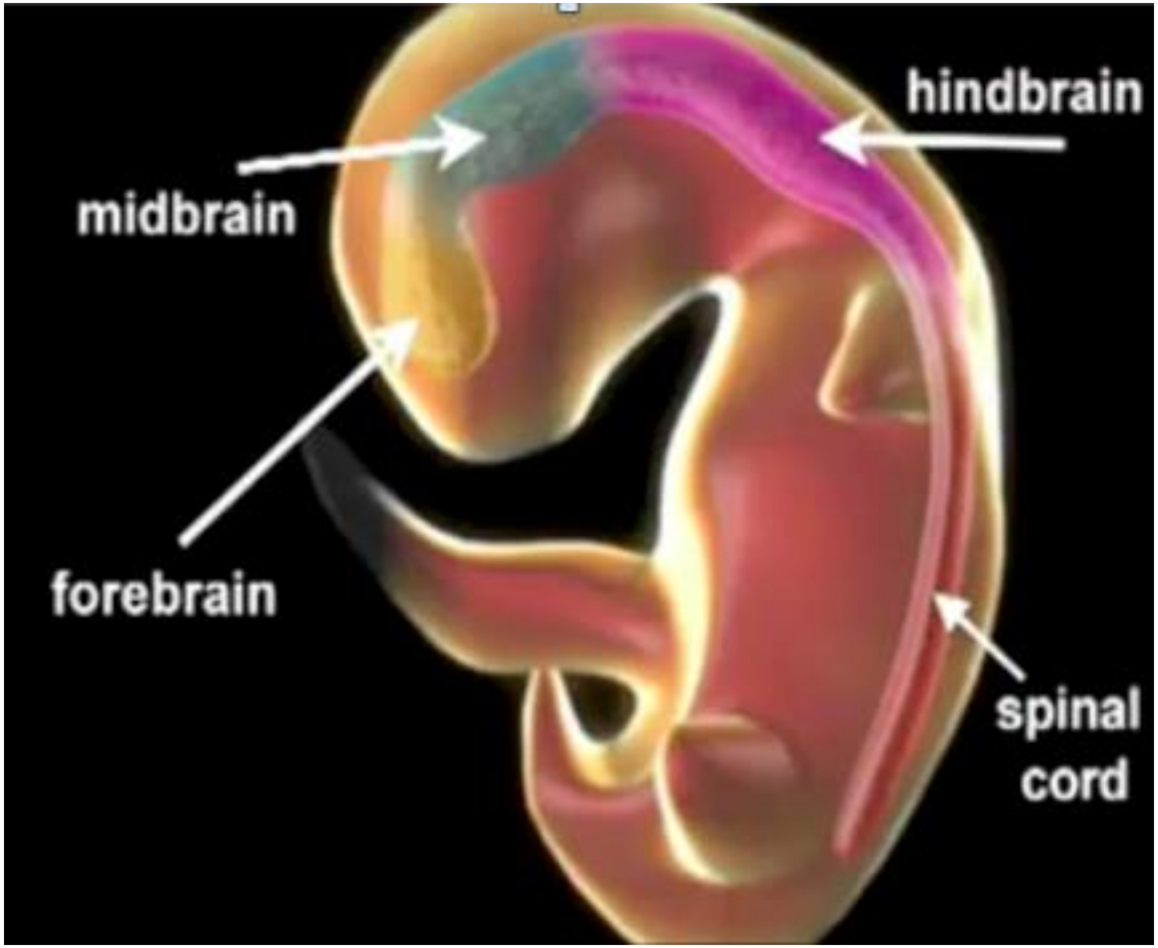
centralia.edu

Neural tube formation



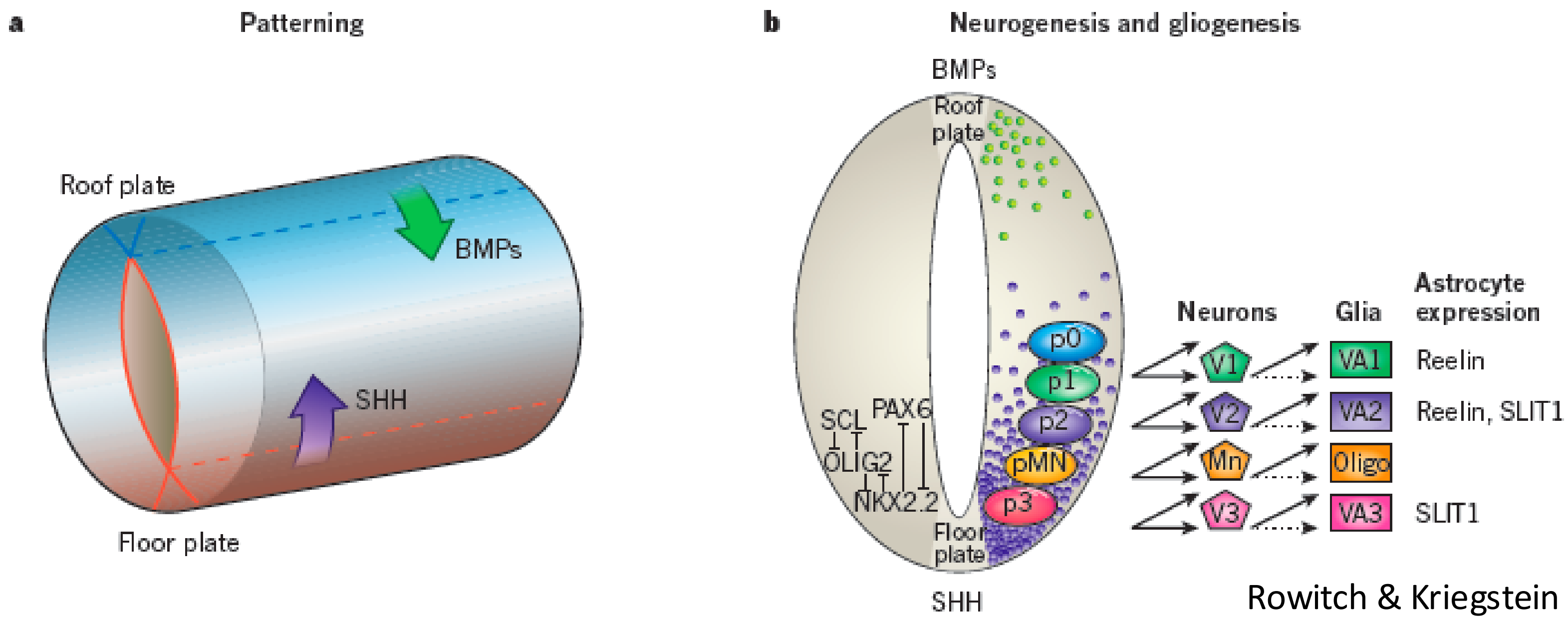
thebrain.mcgill.ca

Patterning occurs already in the neural tube



Patterning occurs already in the neural tube

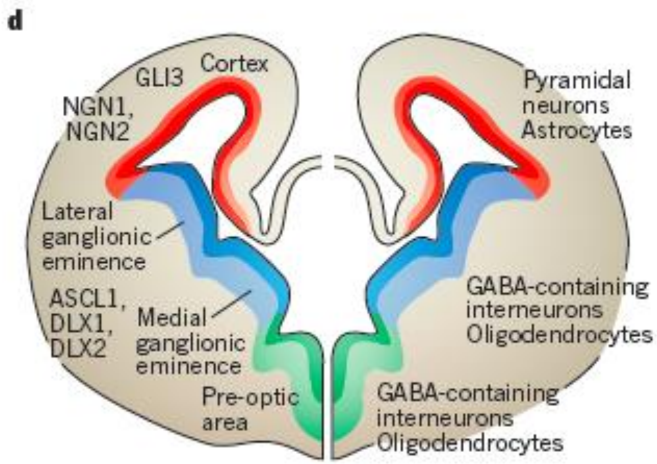
from “simple” and undifferentiated neuroepithelium to a large cellular diversity and specialization in the adult CNS (more than ~200 different types of neurons)



Rowitch & Kriegstein 2010

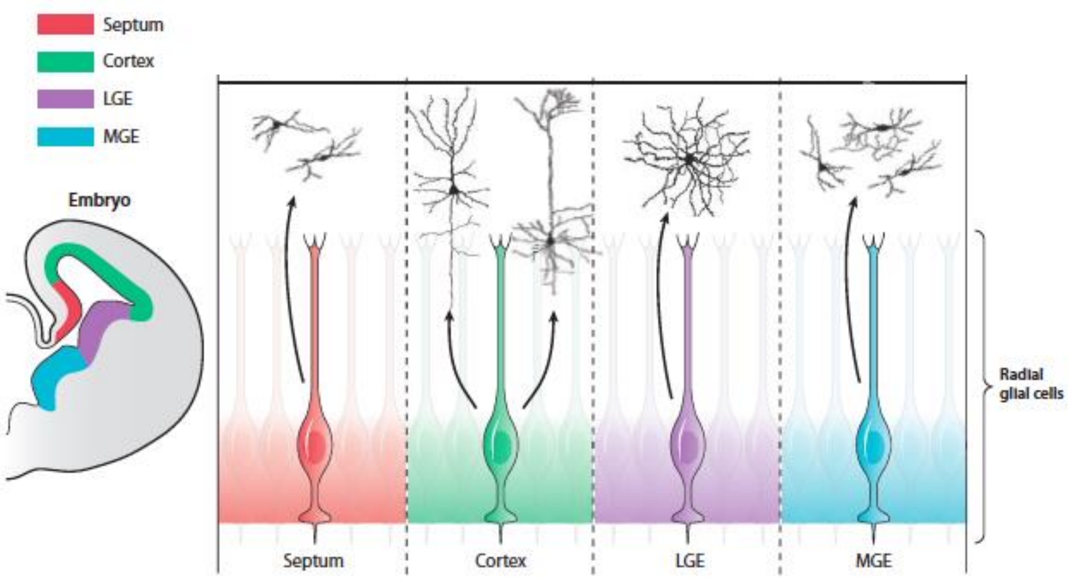
- gradient of morphogenes (e.g. sonic hedgehog and bone morphogenic protein)
- positional identity, segmentation into progenitor domains
- different transcription factors expressed, further specification

Neural stem cells are regionally different



Rowitch & Kriegstein 2010

embryonic forebrain E14.5

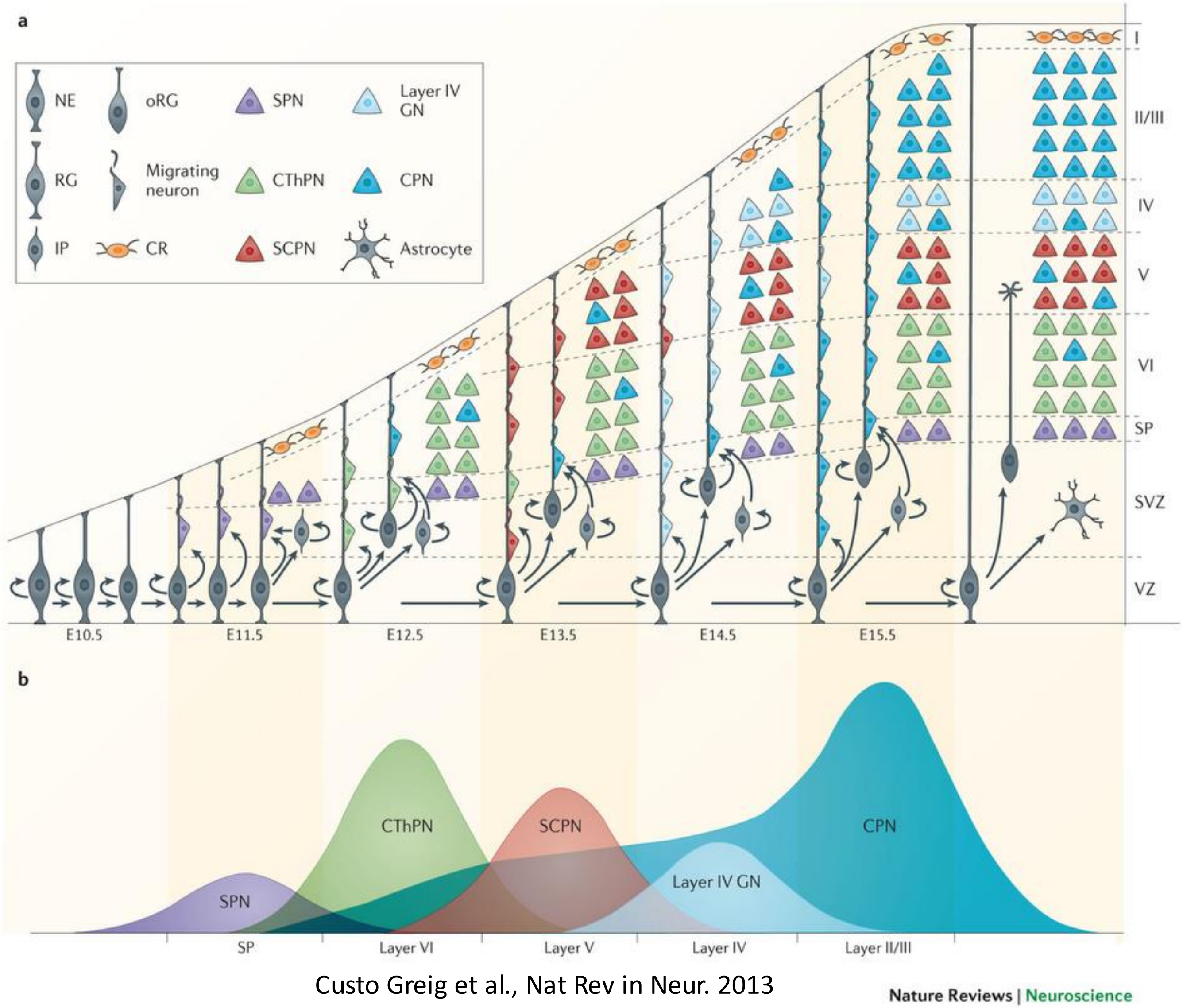


Kriegstein & Alvarez-Buylla, 2009

the term "neural stem cells" comprises regionally different progenitor cells (radial glial cells) that generate different types of neurons

Radial glial cells provide scaffolds for newborn neurons

The cortical layers are formed inside out



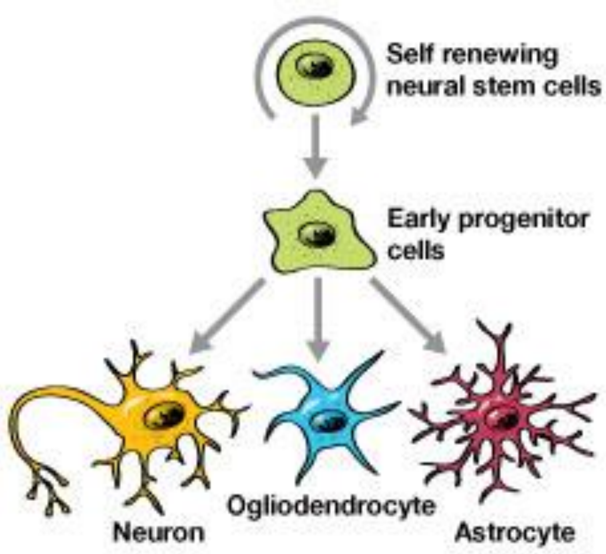
Custo Greig et al., Nat Rev in Neur. 2013

NE: neuroepithelial cells
RG: radial glial cells
SPN: subplate neurons
oRG: outer radial glial cells
IP: intermediate progenitors
CR: Cajal-Retzius cells
CThPN: corticothalamic projection neurons
CPN: callosal projection neurons
SCPN: subcerebral projection neurons
GN: granular neurons

Neural stem cells

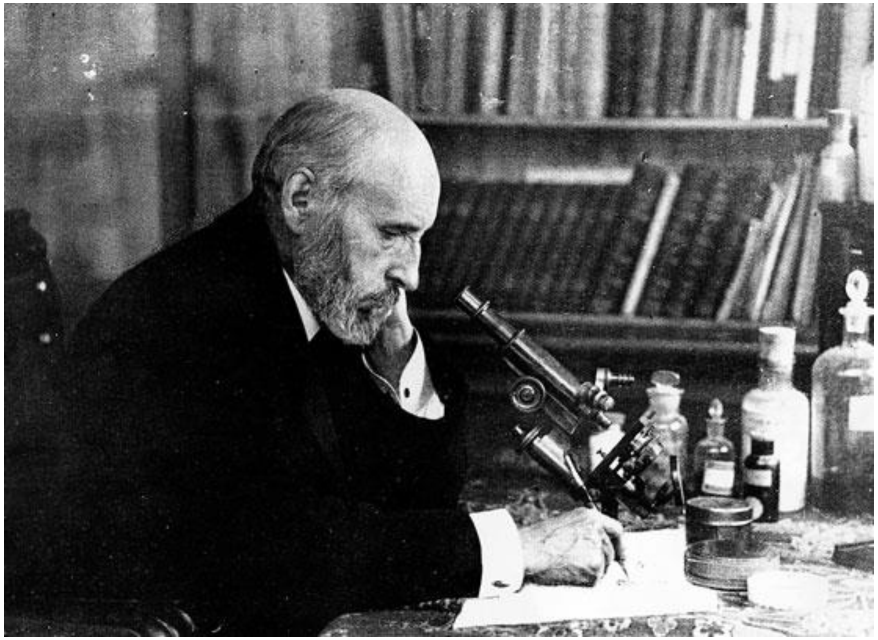
- can self-renew (expansion and maintenance)
- can give rise to neurons and glial cells (astrocytes, oligodendrocytes)

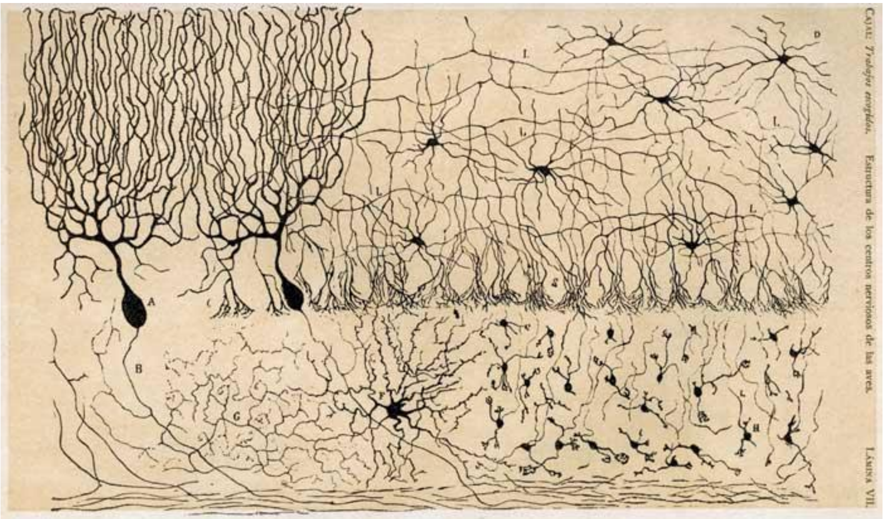
Neurogenesis



other stem cells (for example hematopoietic stem cells) are present **throughout life** and are important for proper body function (blood)

Adult neural stem cells: do they exist?





Santiago Ramon y Cajal (1852-1934)
Spanish pathologist, nobel laureate
"father of modern neuroscience"

"Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably.
In the adult centers, the nerve paths are something fixed, and immutable:
everything may die, nothing may be regenerated."

1928

Evidence of cell division in the adult mammalian brain

Altman and Das, 1964

NATURE December 19, 1964 VOL. 204

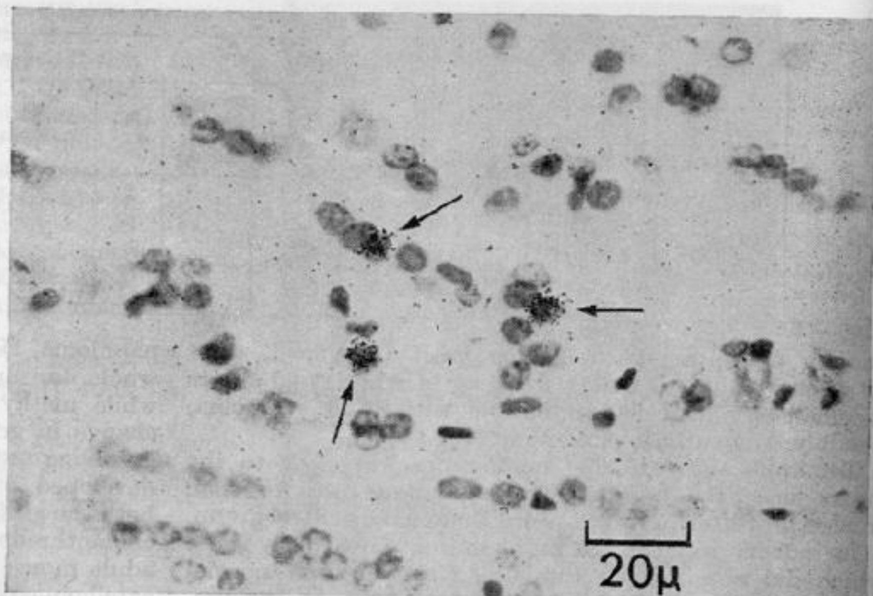


Fig. 1. Photomicrograph of a gallocyanin and chromalum stained autoradiogram. Arrows point to labelled glia cells in cortical radiation

^{3}H-Thymidine labeling in adult rats

Autoradiographic and Histological Evidence of Postnatal Hippocampal Neurogenesis in Rats [1]

JOSEPH ALTMAN AND GOPAL D. DAS

J. Comp. Neurol 1965

Neurogenesis in the Adult Rat: Electron Microscopic Analysis of Light Radioautographs

Kaplan and Hinds, 1977 Science

Songbirds have seasonal changes in brain volume

A Brain for All Seasons: Cyclical Anatomical Changes in Song Control Nuclei of the Canary Brain Nottebohm 1981, Science

Table 1. Ratio of spring to fall measures of brain variables.

Variable	Mean ± standard deviation		P	Spring: fall ratio
	Spring	Fall		
HVc* (mm^3)	0.884 ± 0.243	0.444 ± 0.105	< .001	1.99
RA* (mm^3)	0.519 ± 0.114	0.293 ± 0.058	< .001	1.77
Rt† (mm^3)	0.572 ± 0.056	0.481 ± 0.039	< .001	1.19
SpM† (mm^3)	0.111 ± 0.015	0.099 ± 0.013	> .05	1.12
Caudal forebrain* (mm^3)	7.93 ±0.120	6.47 ± 0.440	< .001	1.23
Brain weight (g)	0.754 ± 0.065	0.655 ± 0.041	< .001	1.15
HVc:Rt	0.764 ± 0.186	0.463 ± 0.118	< .001	1.65
RA:Rt	0.608 ± 0.213	0.385 ± 0.122	< .001	1.58

*Corresponds to volume reconstruction of left and right structures. †Corresponds to volume reconstruction of left structures.

Neurons Generated in the Adult Brain Are Recruited into Functional Circuits

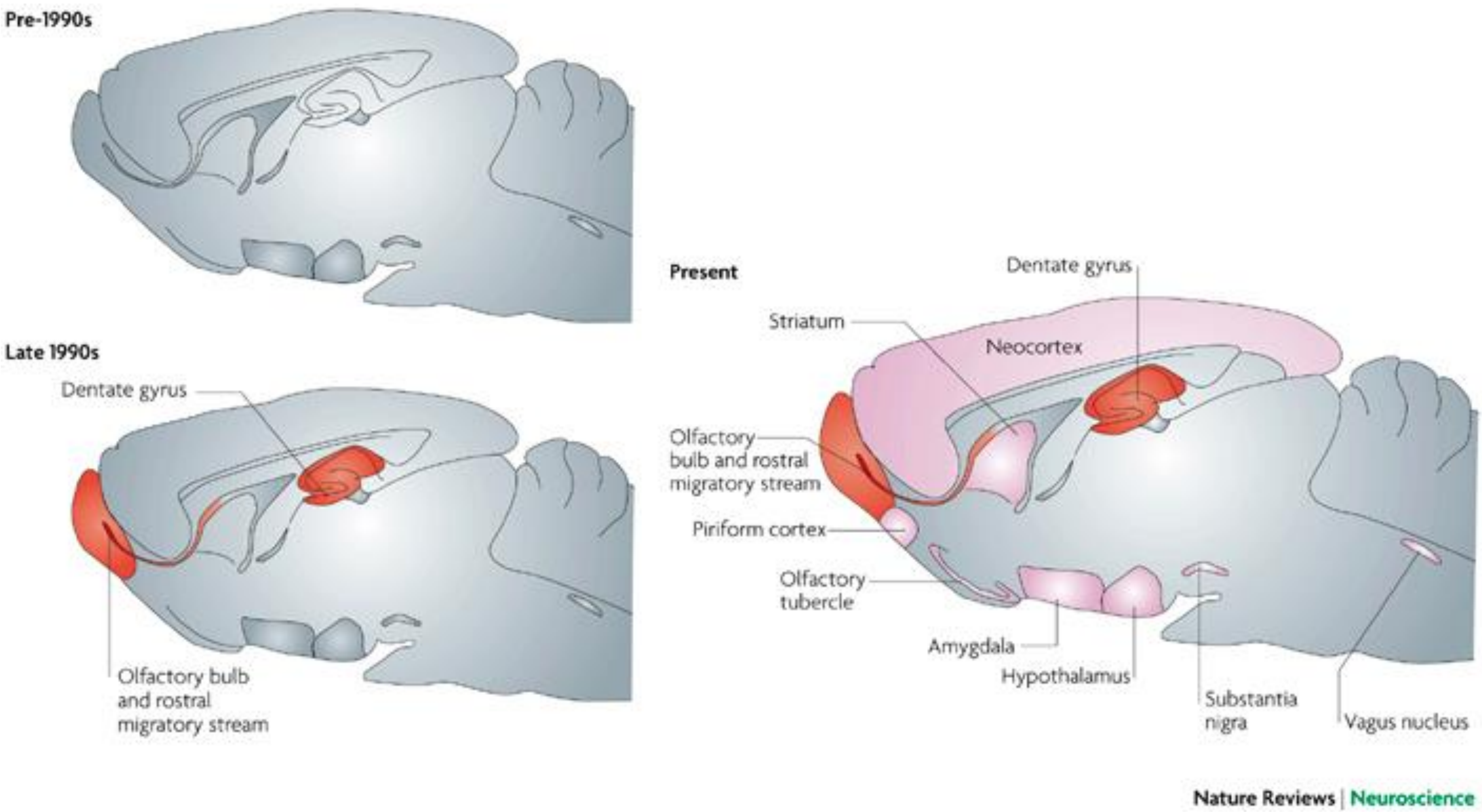
Paton and Nottebohm 1984, Science

Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain

(learning/neurogenesis/neuronal death/glial cells/endothelial cells)

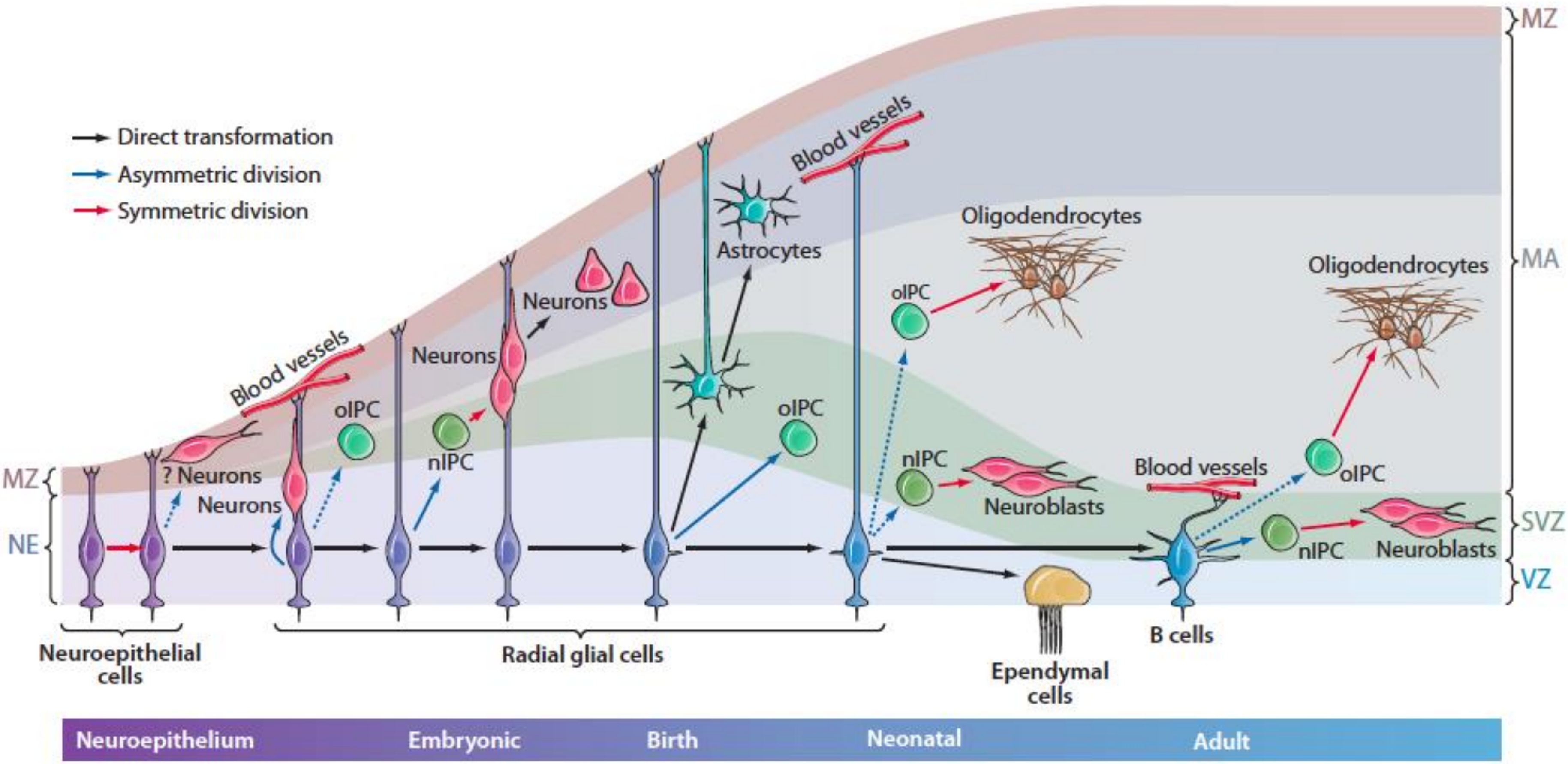
STEVEN A. GOLDMAN AND FERNANDO NOTTEBOHM PNAS 1983

Adult mammalian neurogenesis: at least in 2 distinct regions



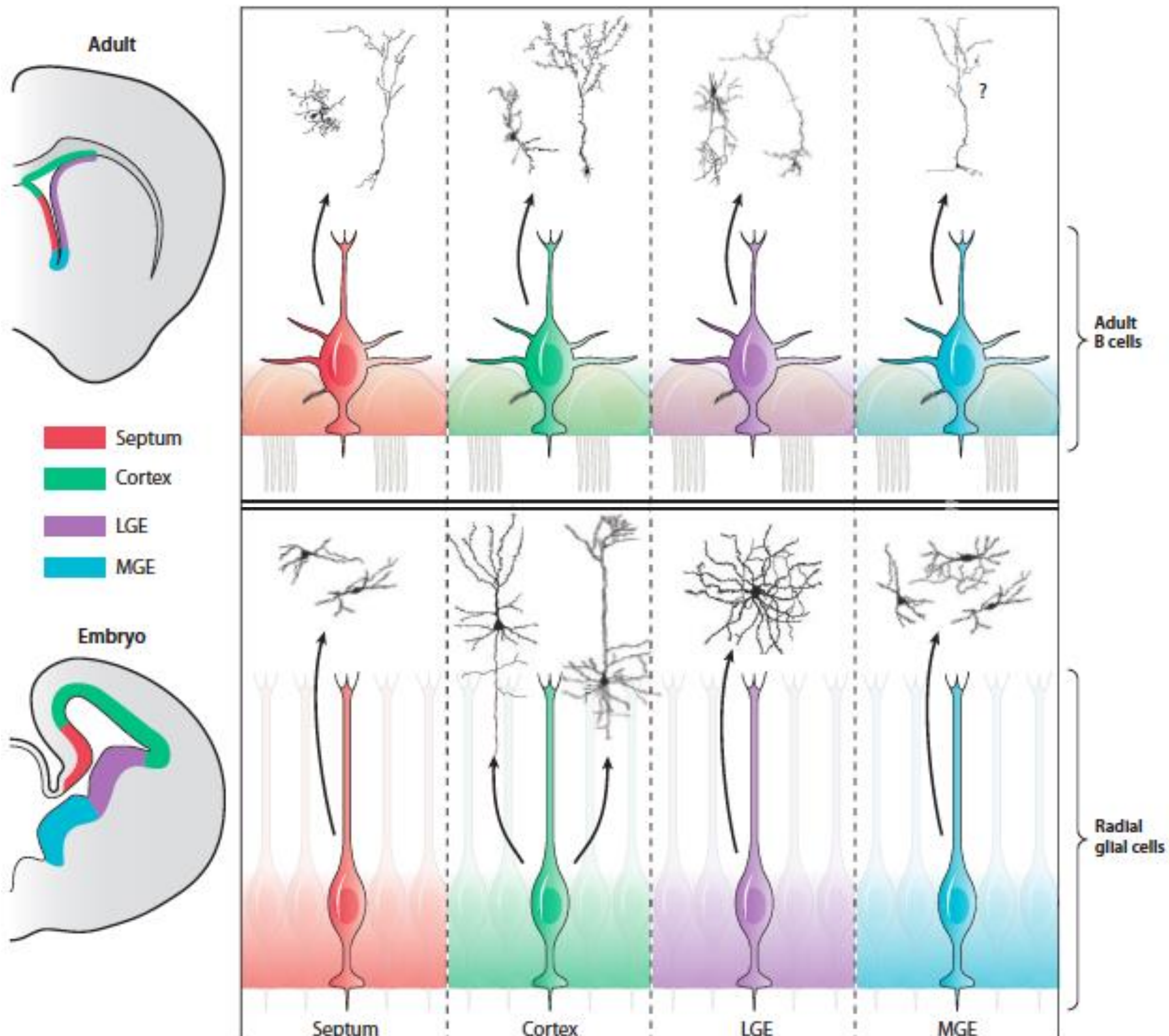
red: confirmed ongoing neurogenesis
pink: controversial evidence, possibly low levels of neurogenesis

Adult neural stem cells: similar to embryonic radial glia



Kriegstein & Alvarez-Buylla, 2009

Adult neural stem cells are regionally different



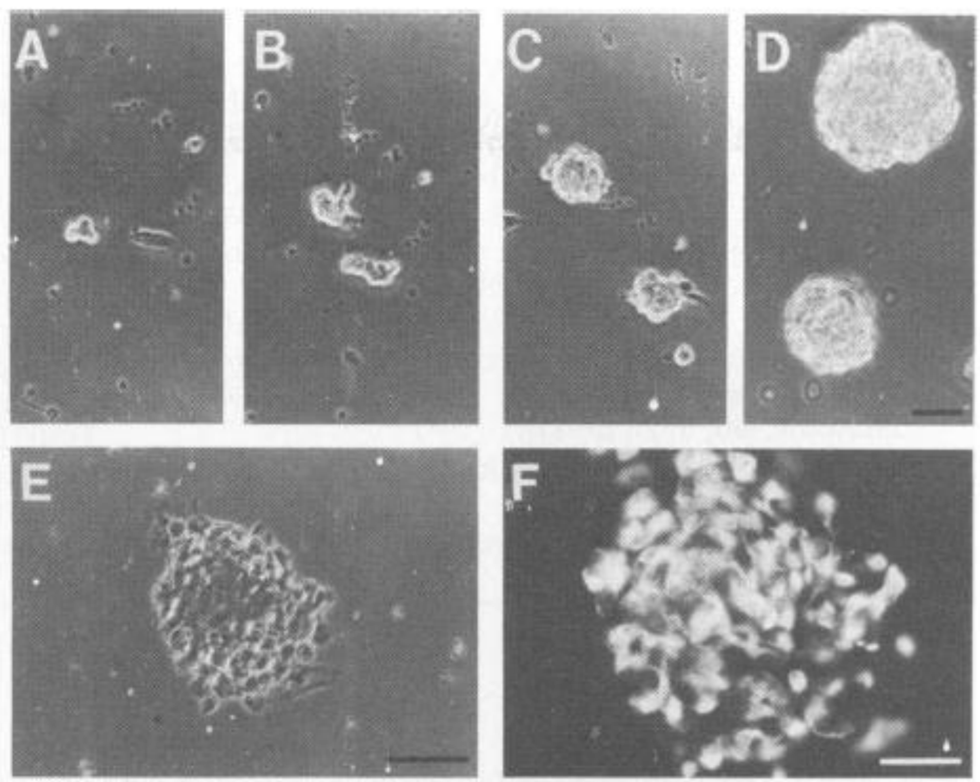
Kriegstein & Alvarez-Buylla, 2009

Adult neural stem cells: first isolation

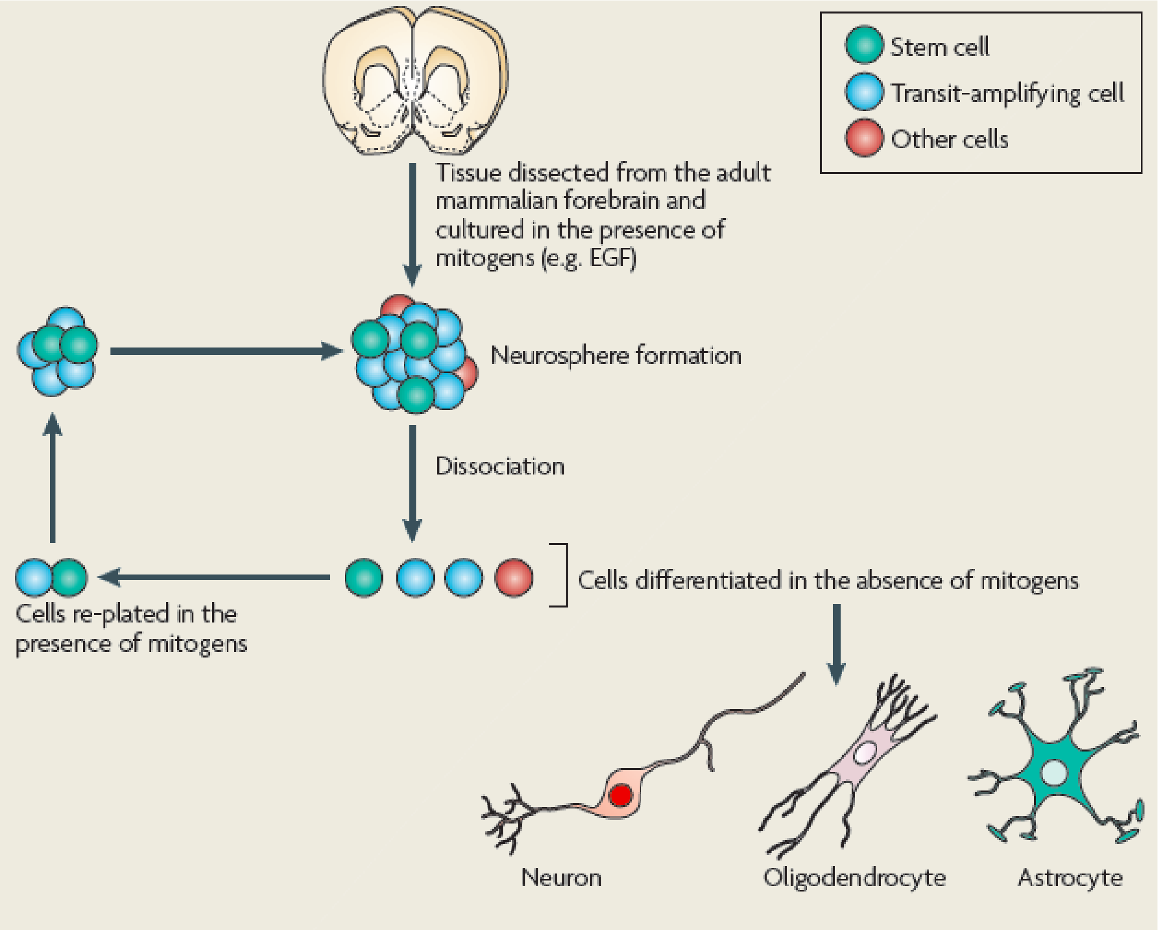
Generation of Neurons and Astrocytes from Isolated Cells of the Adult Mammalian Central Nervous System

BRENT A. REYNOLDS AND SAMUEL WEISS*

SCIENCE, VOL. 255
27 MARCH 1992



Adult neural stem cells: neurospheres and differentiation



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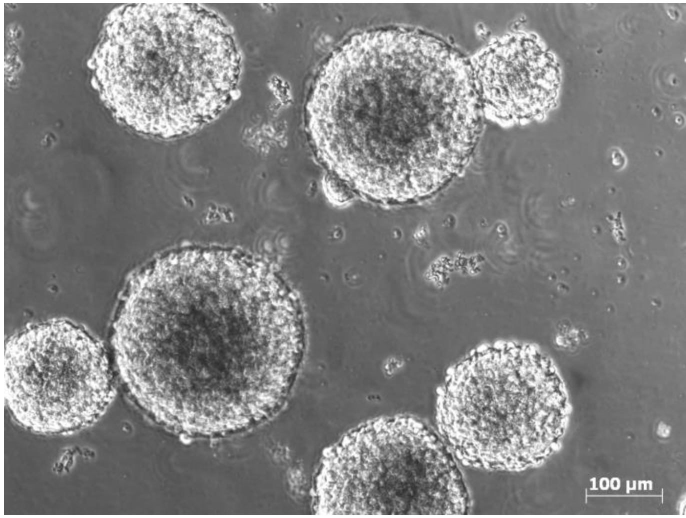
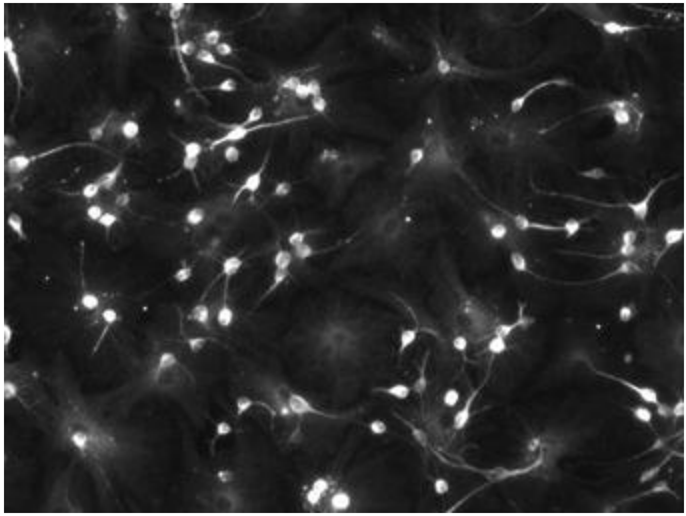
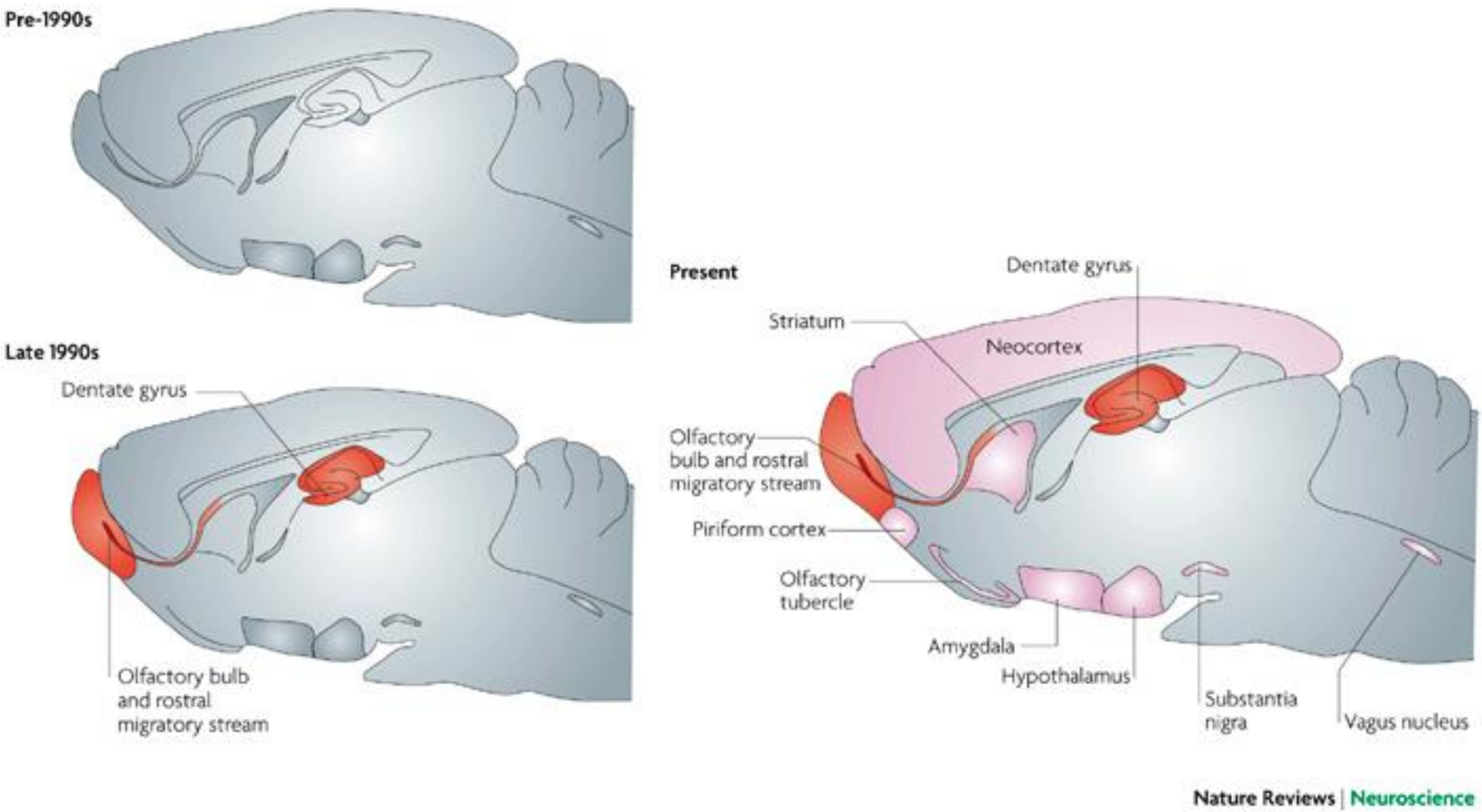
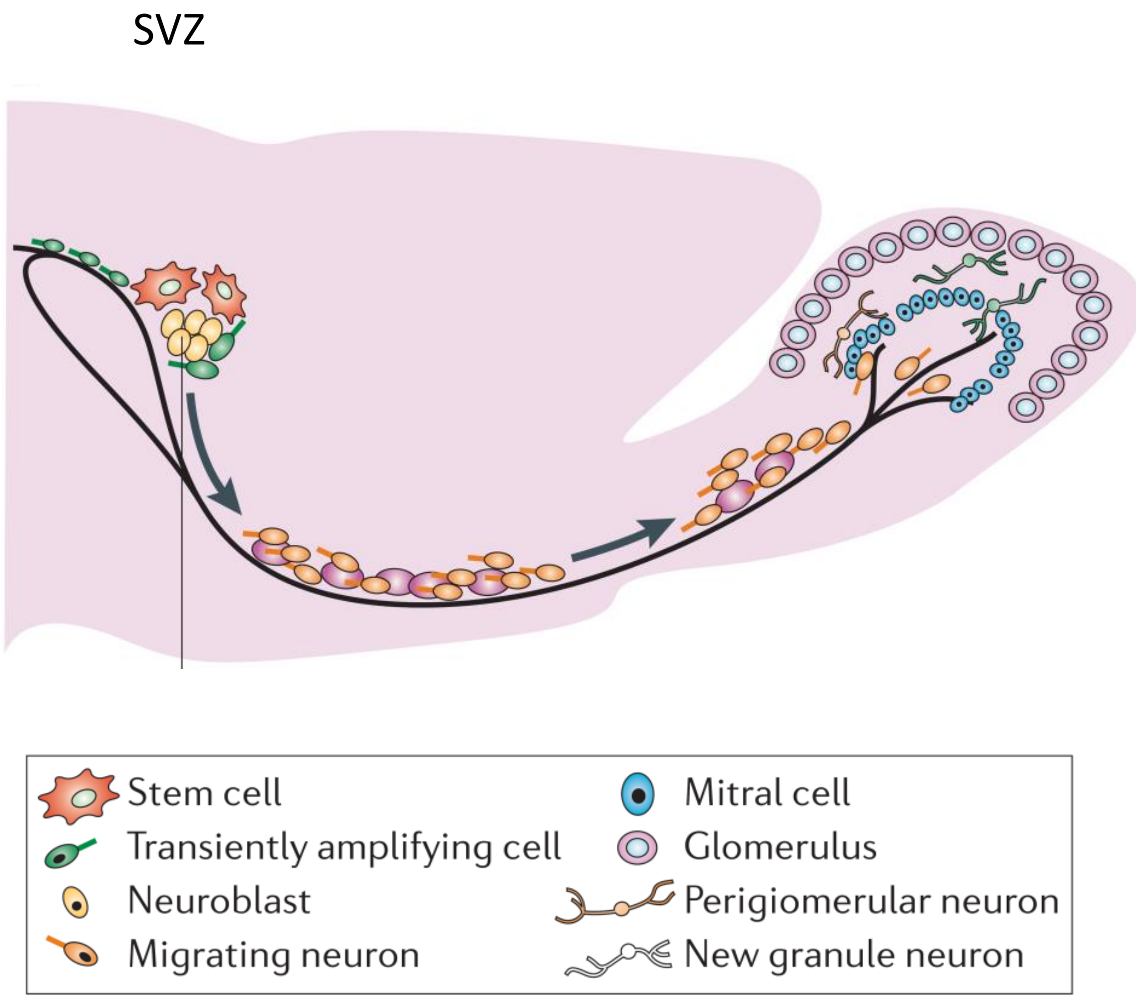




Adult mammalian neurogenesis: at least in 2 distinct regions



Adult neurogenesis: Subventricular zone and dentate gyrus

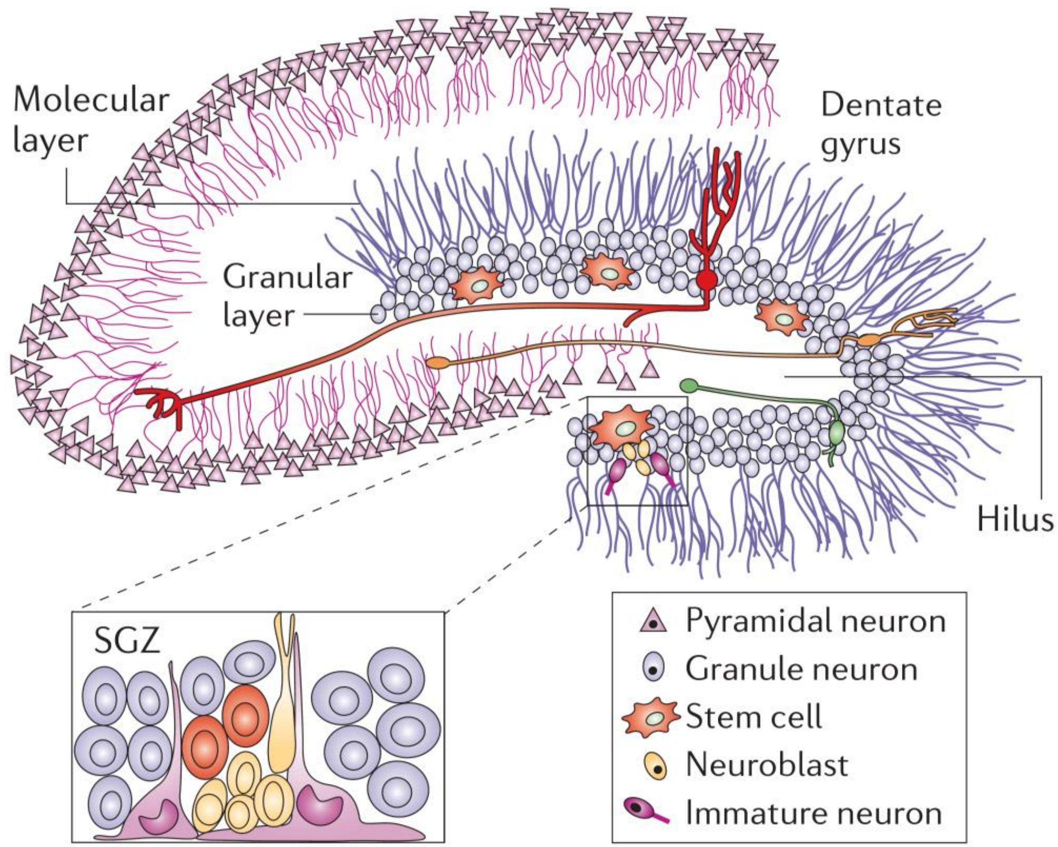


- stem cells reside in the lateral ventricle wall
- progenitors/neuroblasts migrate along rostro-migratory stream towards olfactory bulb
- they differentiate into granule neurons and periglomerular neurons

Modified from Vescovi et al., 2006

Adult neurogenesis: subventricular zone and dentate gyrus

DG



Vescovi et al., 2006

- stem cells reside in the subgranular zone of the DG
- newborn neurons mature into granule neurons
- only migrate a short distance
  into granular layer

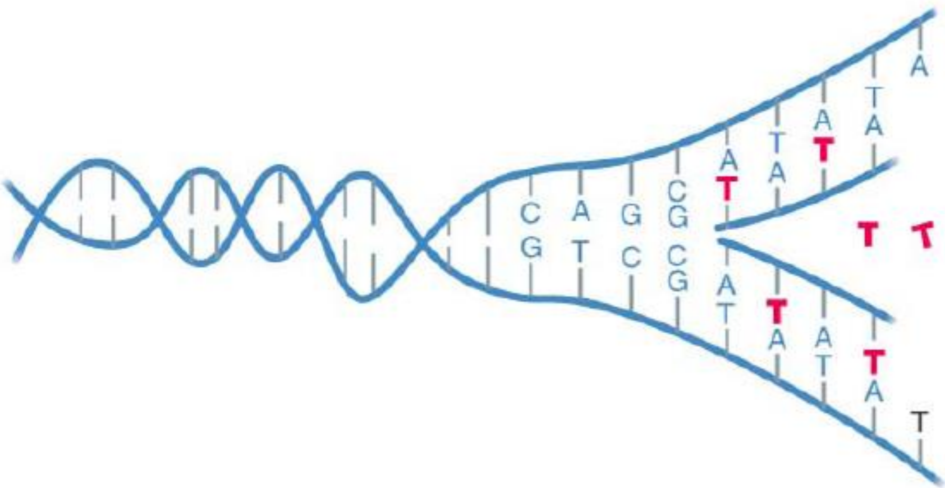
newborn neurons are initially more excitable than adult neurons (lower threshold to react to stimulus)

Why does neurogenesis occur only in these two regions?

- instructive cues?
- aversive cues in other regions? (active repression?)
- “Niche”-concept (specialized microenvironment needed?)
- only there neural stem cells left?

➡ remains to be resolved

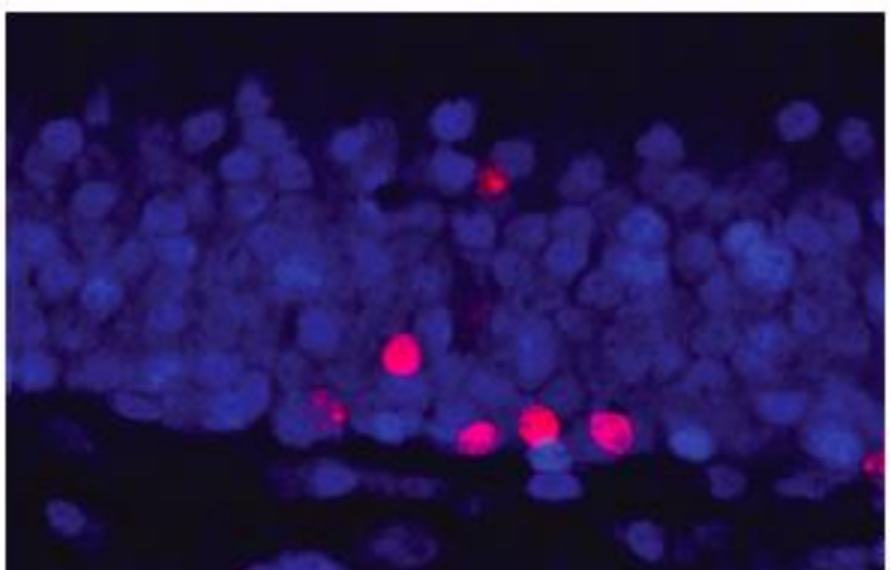
Tools to study adult neurogenesis: labeling proliferating cells



(Modified from Angevine, 1970.)

^{3}H Thymidine — radioactive (autoradiography)

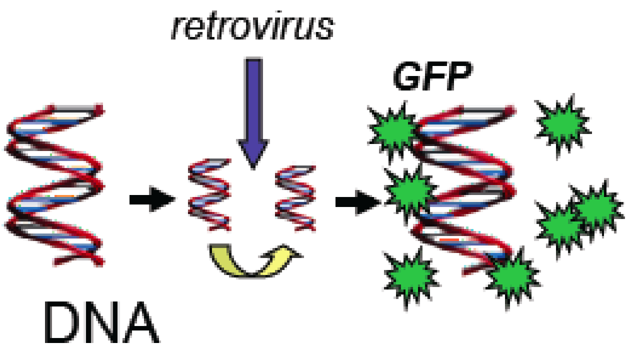
BrdU
EdU — via Antibody staining

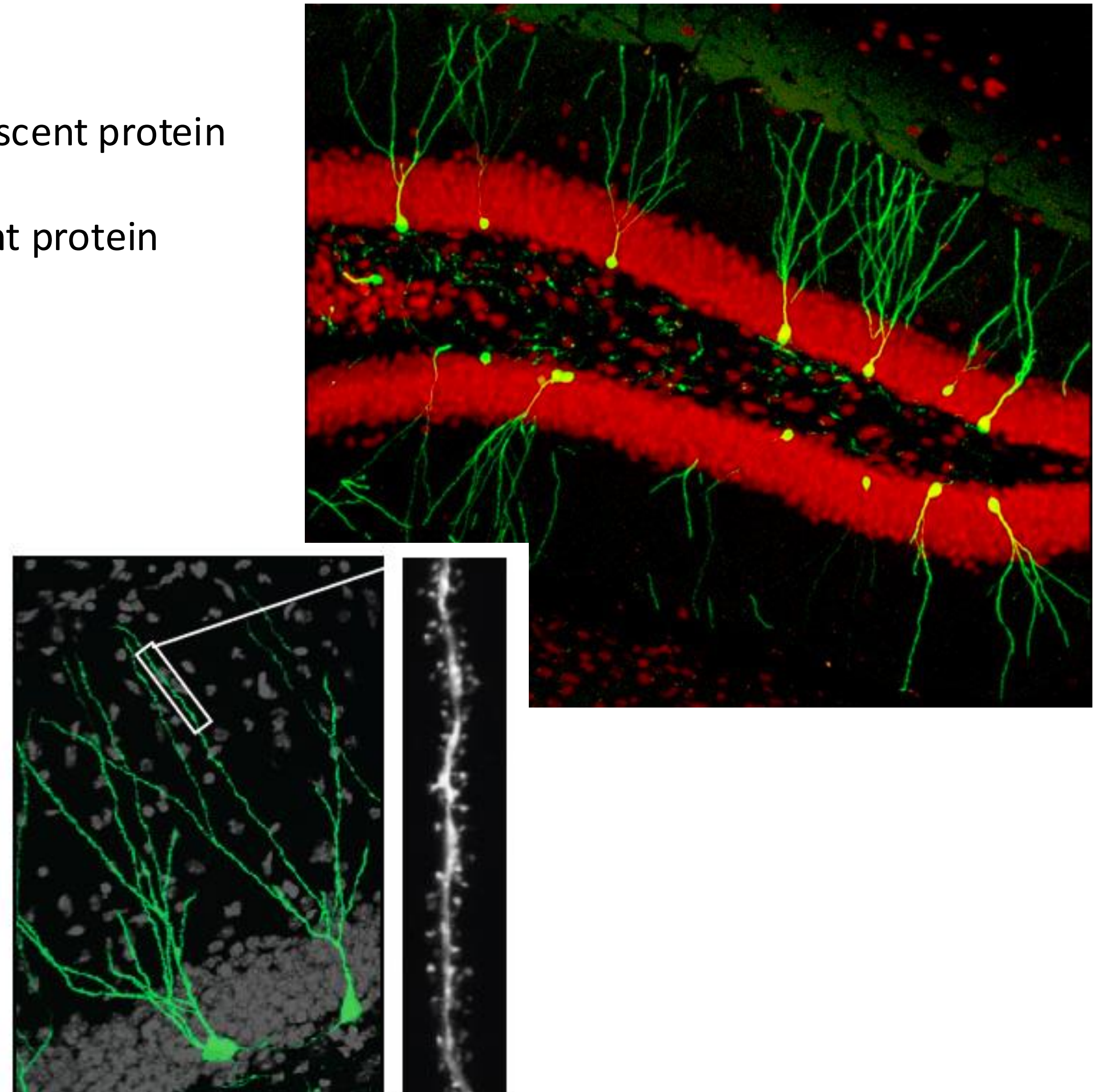


BrdU / NeuN

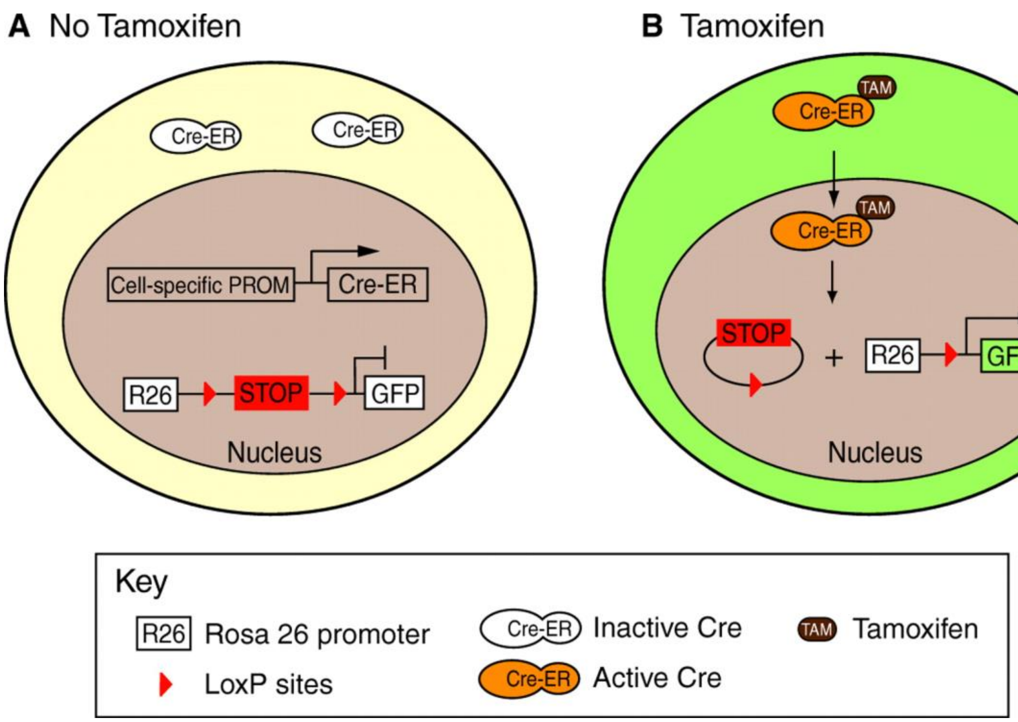
Tools to study adult neurogenesis: labeling proliferating cells

Retrovirus encoding eg. for fluorescent protein
can only integrate in dividing cells
all progeny express the fluorescent protein

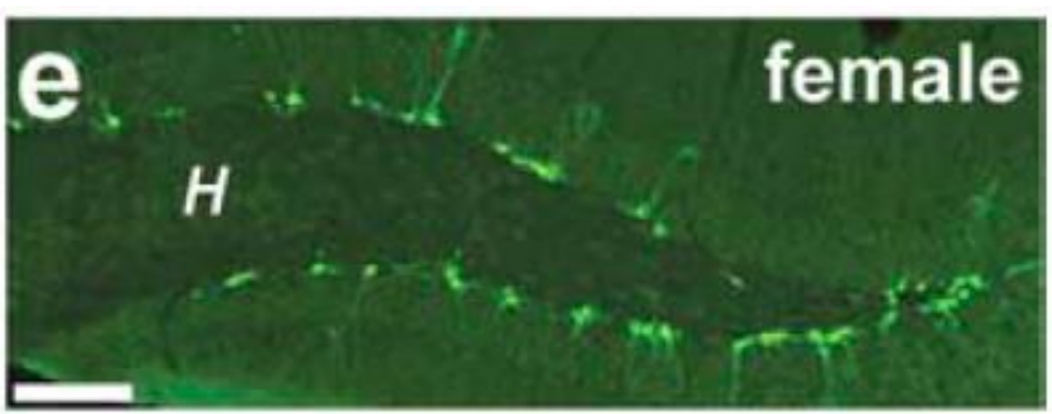




Tools to study adult neurogenesis: genetic lineage tracing

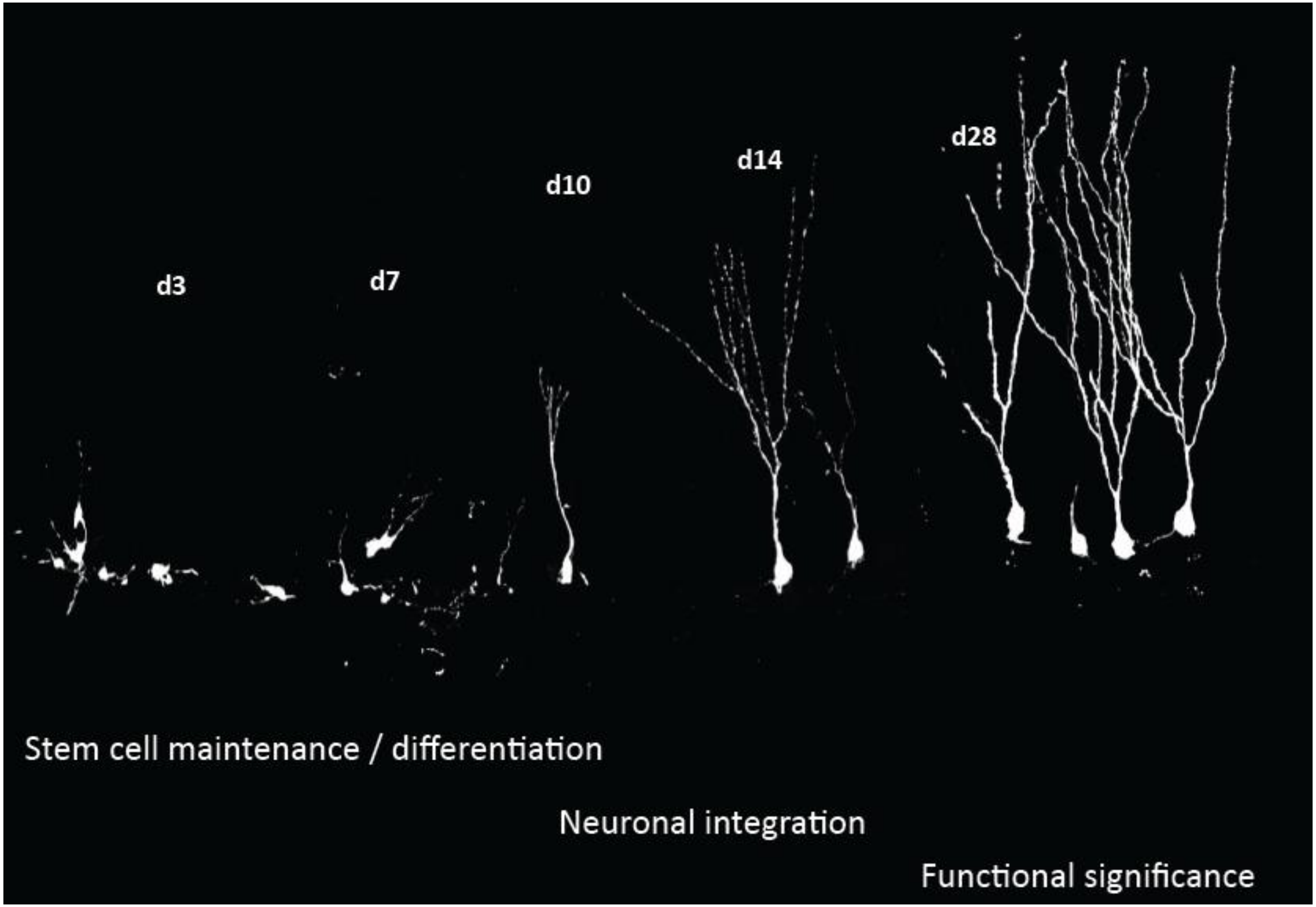


dev.biologist.org



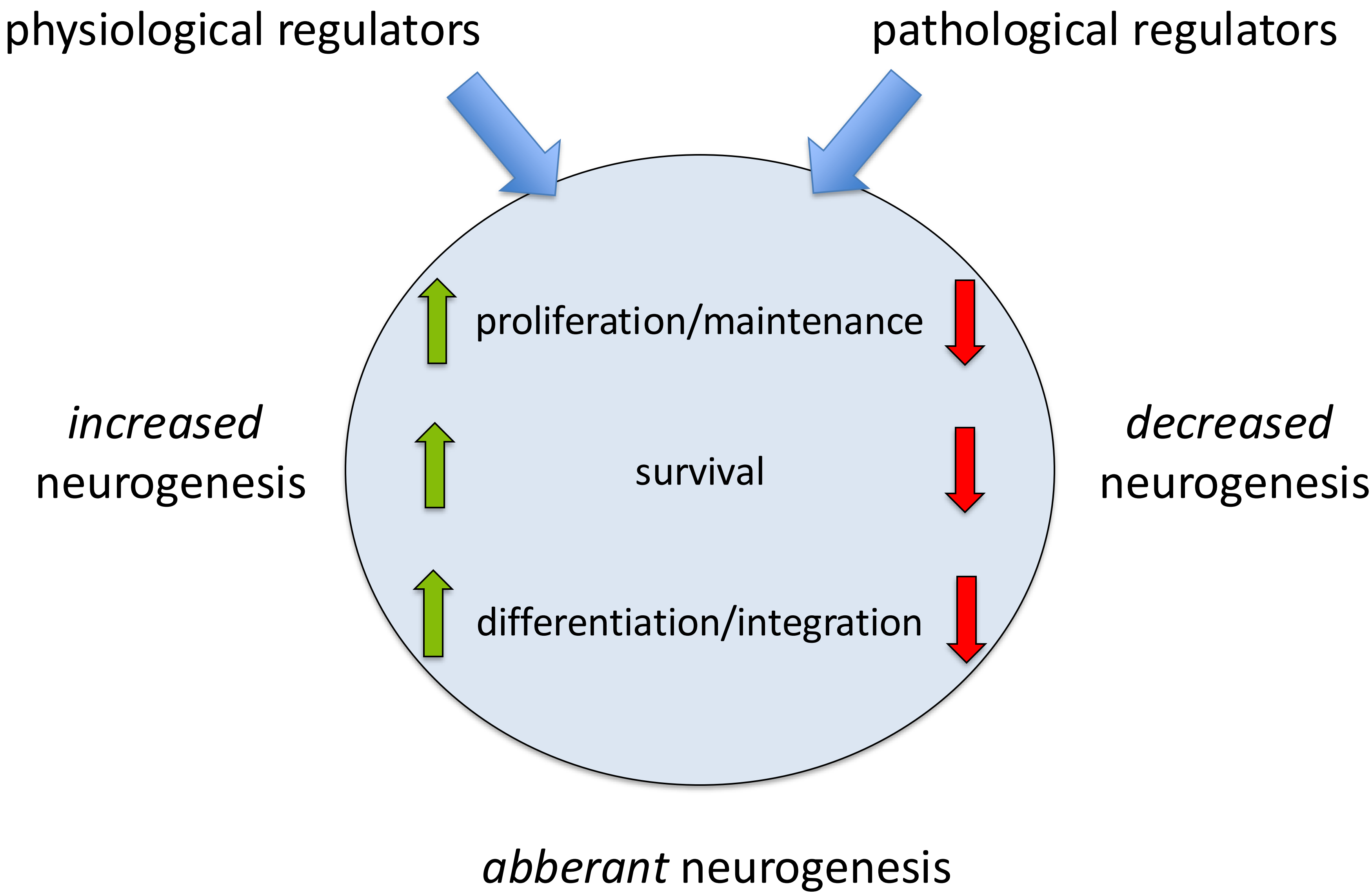
Lagace et al. 2007

Adult neurogenesis: a complex process



all these stages at the same time in the adult brain in relative close proximity

What regulates adult neural stem cells:



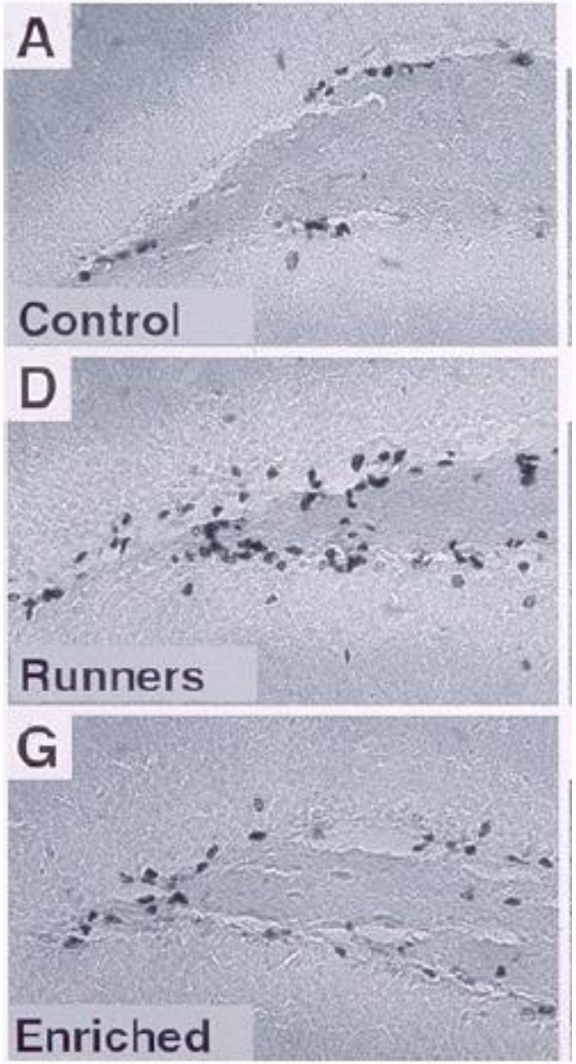
What regulates adult neural stem cells:

TABLE 7.1 Regulators of adult neurogenesis

Regulator	Proliferation	Survival	Neuronal Differentiation
Physiological Regulators of Adult Neurogenesis			
Genetic background	+/−	+/−	+/−
Enriched environment	?	+	+
Physical exercise	+	no change	+
Learning	no change	+	?
Aging	−	?	−
Dietary restriction	no change	+	?
Neurotransmitters	see Text		
Pathological Regulators of Adult Neurogenesis			
Stress	−	no change	?
Seizure activity	+	+(?)	+
Ischemia	+	+(?)	+
Irradiation	−	−	−
Neurodegenerative Diseases			
Alzheimer's Disease	+/−	?	?/−
Huntington's Disease	−	no change	no change
Parkinson's Disease	−	?	?
Drugs			
Opiates	−	?	?
Antidepressants	+	+	+
Ethanol	no change/−	−	?

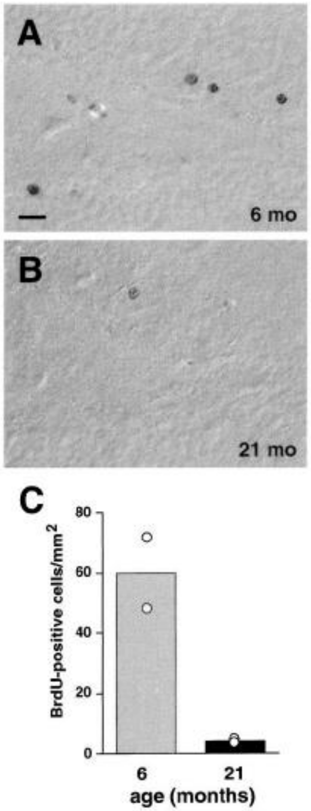
Jessberger et al.2008
CNS Regeneration: Basic Science and Clinical Advances, Academic Press

Physiological regulators: running and enriched environment

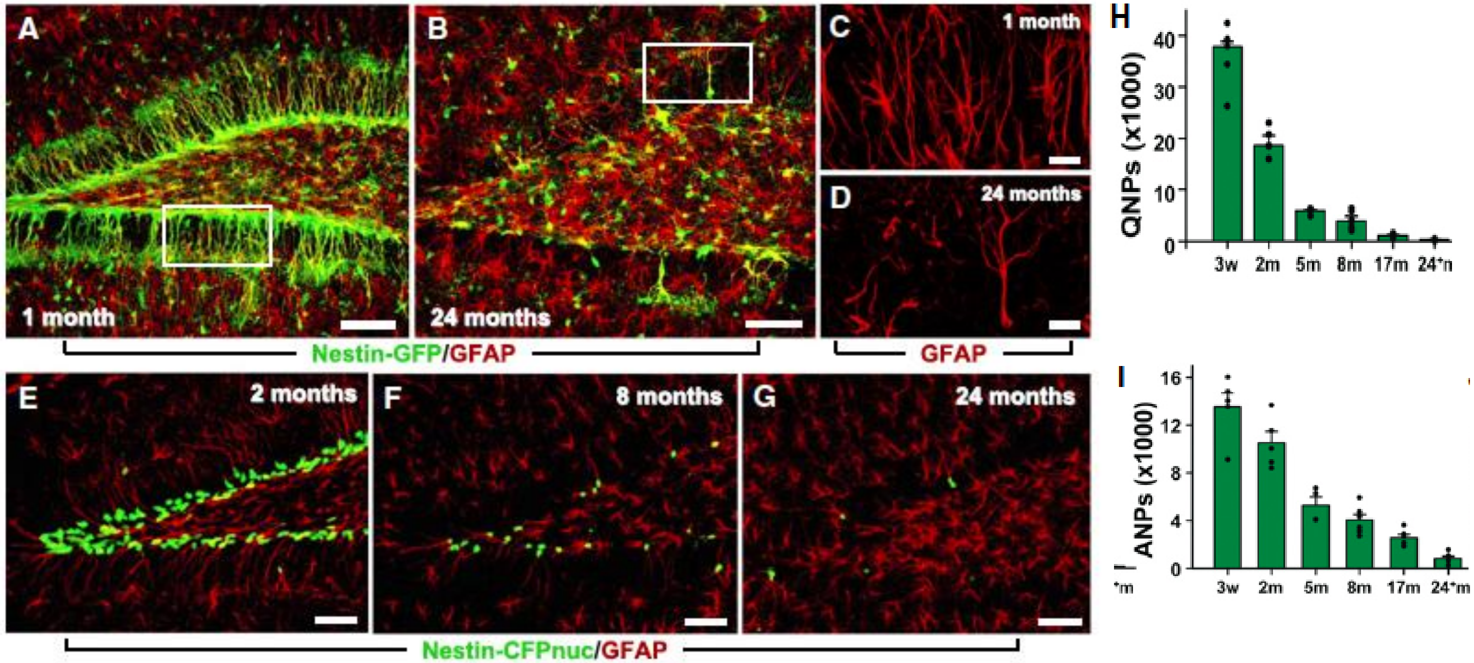


Kempermann et al. 1997
van Praag et al. 1999

Physiological regulators: aging decreases neurogenesis



Kuhn et al. 1996 J. Neurosci.



Encinas et al. 2011 Cell Stem Cell

- exhaustion of adult neural stem cells?
- not enough proliferative stimuli, i.e. more quiescent?
- aversive stimuli?

Blood borne factors might be one reason for this decline

90 | NATURE | VOL 477 | 1 SEPTEMBER 2011

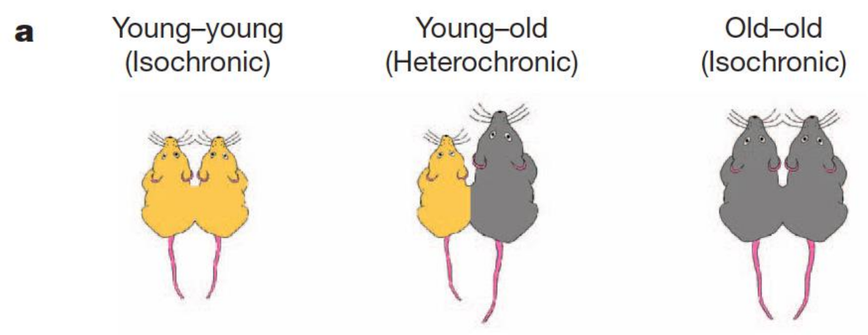
LETTER

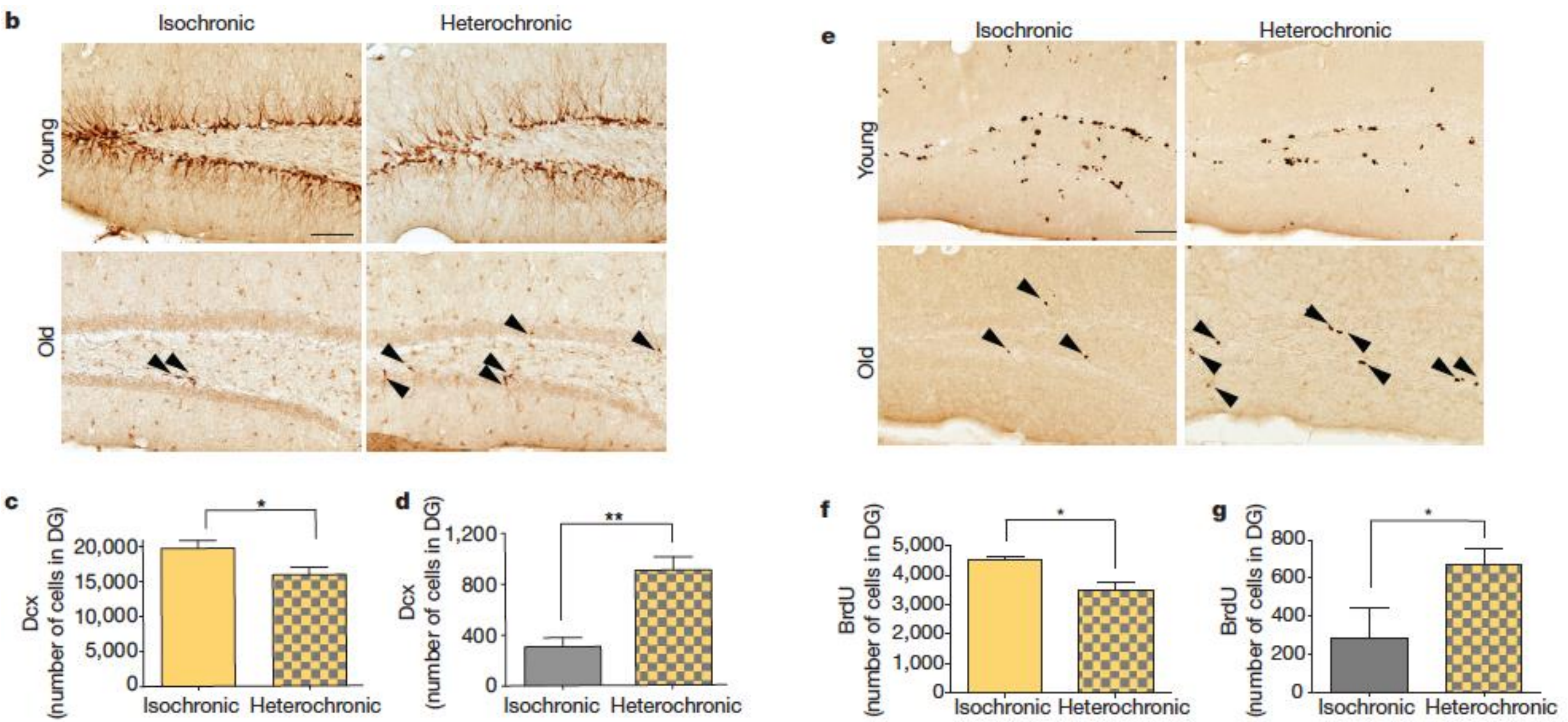
doi:10.1038/nature10357

The ageing systemic milieu negatively regulates neurogenesis and cognitive function

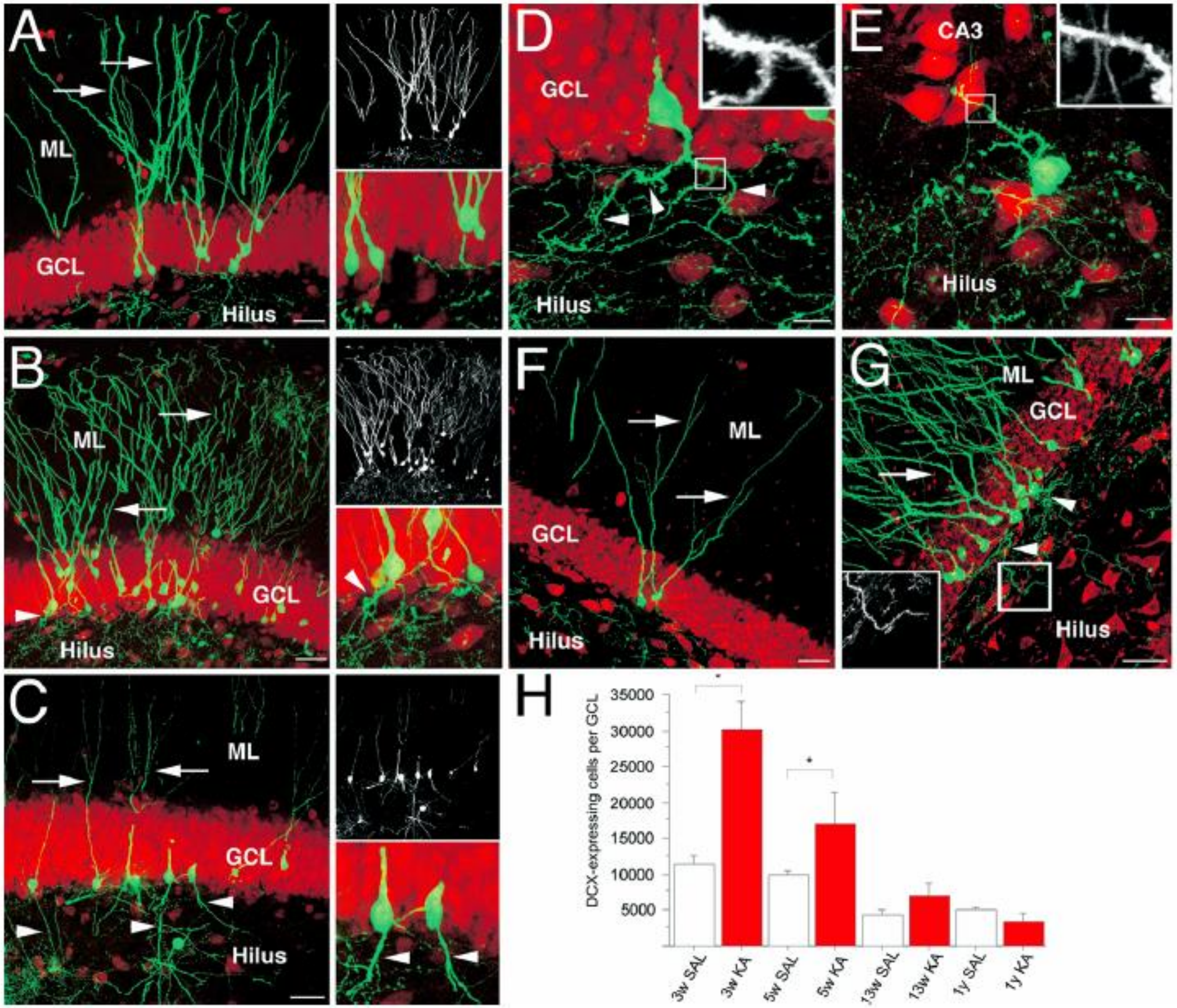
Saul A. Villeda[1,2], Jian Luo[1], Kira I. Mosher[1,2], Bende Zou[3], Markus Britschgi[1]†, Gregor Bieri[1,4], Trisha M. Stan[1,5], Nina Fainberg[1], Zhaoqing Ding[1,5], Alexander Eggel[1], Kurt M. Lucin[1], Eva Czirr[1], Jeong-Soo Park[1]†, Sebastien Couillard-Després[6], Ludwig Aigner[6], Ge Li[7], Elaine R. Peskind[7,8], Jeffrey A. Kaye[9], Joseph F. Quinn[9], Douglas R. Galasko[10], Xinmin S. Xie[3], Thomas A. Rando[1,11,12] & Tony Wyss-Coray[1,2,5,11]

Parabiosis = coupling the blood circulation



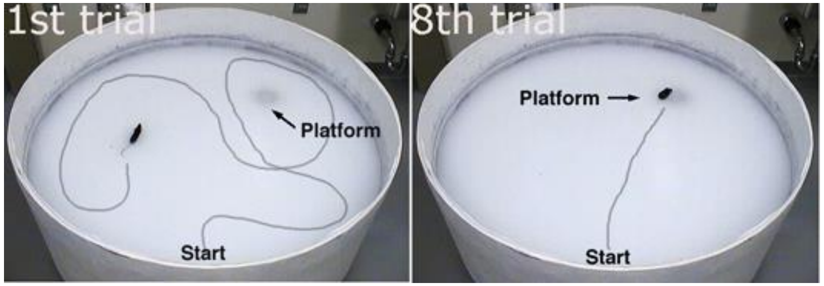


Pathological regulators: seizures/epilepsy



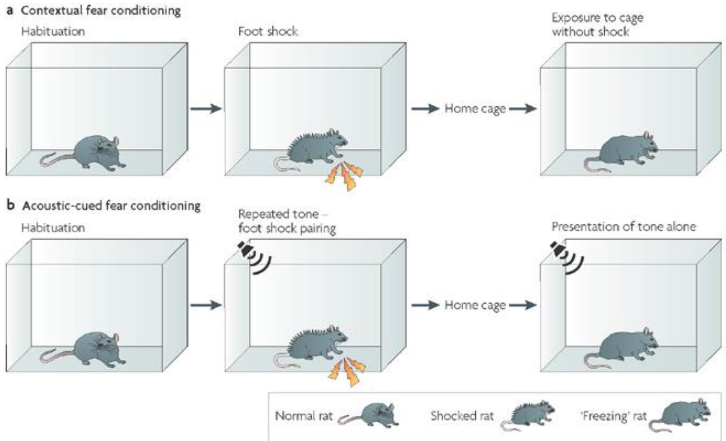
Jessberger et al. 2007 J. Neurosci.

Tools to study adult neurogenesis: behavioral tests



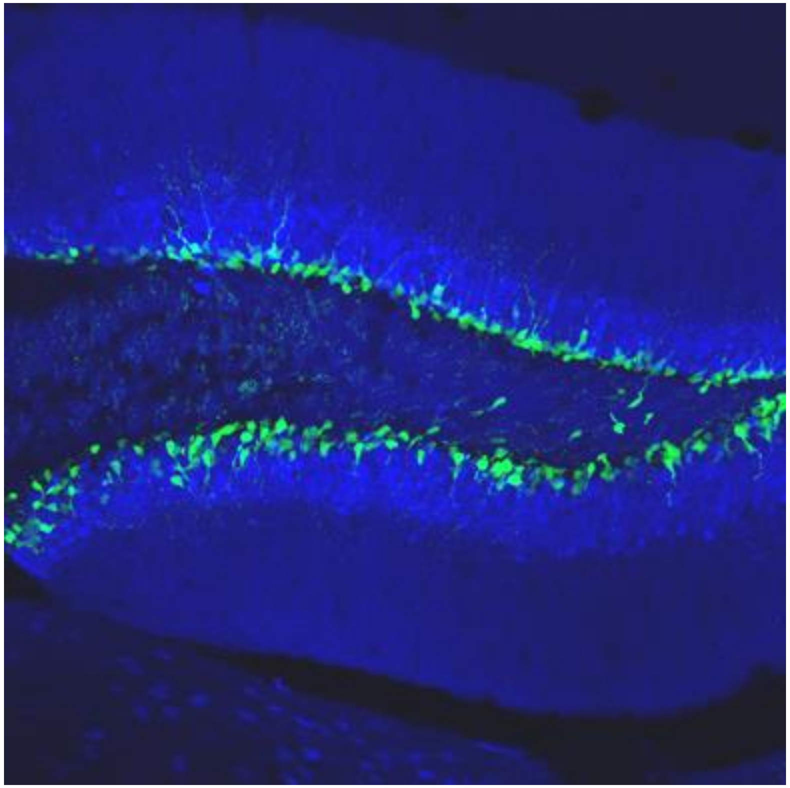
Morris Water Maze: spatial learning and memory

radial arm maze: spatial learning and memory



Contextual fear conditioning: associative memory

Problem: all these tests assess hippocampal functions, but the newborn neurons are only a very small fraction of all the hippocampal neurons….sensitive enough?



green POMC (newborn neurons)
blue Calbindin (mature neurons)

Tools to study adult neurogenesis: ablation of neurogenesis

Approach:	***Disadvantage:***
Cytostatic drugs	affects all proliferating cells/side effects
Irradiation	Unspecific effect of x-ray (inflammation, effect on postmitotic cells)
Local cell ablation (e.g. lentiviral vectors)	Which genes are specific? Traumatic injections
Transgenic mice (using TK or suicide genes under the control of stem cell promoters)	Unspecificity / many dead cells at once (e.g. expression of nestin and GFAP in skin/intestines)

Functional significance: controversial results (behaviour)

Behavioural tasks	Ablation method	Phenotype	References
Morris Water Maze			
hidden platform	irradiation	no effect on long-term retention	Synder et al., 2005
hidden platform	irradiation	no effect on long-term retention	Synder et al., 2005
hidden platform followed by reversal	Lentivirus-dnWnt	no effects on acquisition, short term retention or reversal learning, deficit in long-term retention	Jessberger et al., 2009
visible platform followed by hidden platform	irradiation	no effects on acquisition	Saxe et al., 2006
hidden platform	irradiation	no effects on acquisition, deficit in short-term retention	Rola et al., 2004
hidden platform	irradiation	no effects on acquisition or short-term retention	Raber et al., 2004
visible platform followed by hidden platform	Genetic, Dox induced	deficits in acquisition and memory retention	Dupret et al., 2008
hidden platform followed by reversal	Genetic, TM induced	deficits in acquisition, reversal learning and retention	Zhang et al., 2008
hidden platform followed by reversal	Genetic, Dox induced	deficits in acquisition, reversal learning and retention	Farioli-Vecchioli et al., 2008
hidden platform	Genetic, GCV induced	no effects on acquisition and short term retention	Deng et al., 2009
hidden platform	Genetic, GCV induced	no effects on acquisition, short-term retention or long-term retention	Deng et al., 2009
hidden platform	Genetic, GCV induced	no effects on acquisition, short-term retention or long-term retention	Deng et al., 2009
Contextual Fear Conditioning			
10 tone-shock pairs in trace paradigm following pre-exposure	MAM treatment for 14 days	no effect on mobility	Shors et al., 2002
10 tone-shock pairs	irradiation	deficits in freezing	Winocur et al., 2006
3 tone-shock pairs	irradiation	deficits in freezing	Warner-Schmidt et al., 2008
7 tone-shock pairs	irradiation	no effect on freezing	Snyder et al., 2009
3 tone-shock pairs	irradiation	deficits in freezing	Saxe et al., 2006
7 tone-shock pairs	irradiation	no effect on freezing	Snyder et al., 2009
7 tone-shock pairs	irradiation	no effect on freezing	Snyder et al., 2009
one unsignalled shock	MAM treatment for 14 days	no effects on contextual conditioning or extinction	Ko et al., 2009
5 tone-shock pairs	Genetic, GCV induced	deficits in freezing	Saxe et al., 2006
3 tone-shock pairs	Genetic, TM induced	deficits in freezing	Imayoshi et al., 2008
2 shocks (unpaired)	Genetic, Dox induced	no effect on freezing	Dupret et al., 2008
1 tone-shock pairs	Genetic, Dox induced	deficits in freezing	Farioli-Vecchioli et al., 2008
1 shock	Genetic, GCV induced	no effect on contextual conditioning, deficits in extinction	Deng et al., 2009
1 shock	Genetic, GCV induced	no effects on contextual conditioning or extinction	Deng et al., 2009
Working Memory			
cued WM: delayed non-match to sample	irradiation	deficits at long but not short delays	Winocur et al., 2006
radial maze:low memory load/high interference	irradiation	no effect	Saxe et al., 2007
radial maze: high memory load/no interference	irradiation	improvement at long but not short delays	Saxe et al., 2007
radial maze: low memory load/limited interference	irradiation	improvement at relatively long delay	Saxe et al., 2007
radial maze: low memory load/limited interference	Genetic, GCV induced	improvement at long but not short delays	Saxe et al., 2007
radial maze: working memory	Genetic, Dox induced	deficits in working memory	Farioli-Vecchioli et al., 2008
Object Recognition Memory			
object exploration in an arena	irradiation	no effect	Madsen et al., 2003
object exploration in an arena	irradiation	no effect	Madsen et al., 2003
object exploration in an arena	Lenti-viral dnWnt	deficits in long-term retention	Jessberger et al., 2009
object exploration in an arena	irradiation	no effect	Rola et al., 2004
touch screen: object-place association	irradiation	no effect	Clelland et al., 2009
object exploration in an arena	MAM treatment for 14 days	no effect on retention, deficits in enrichment induced long-term retention	Bruel-Jungerman et al., 2005

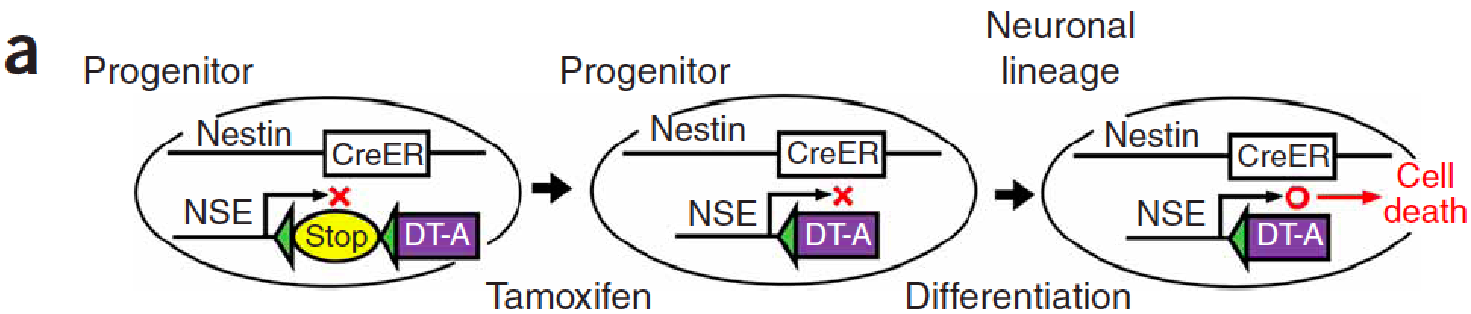
Adapted from Deng et al., 2010 Nature Rev Neuro

Functional significance of adult neurogenesis

Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain

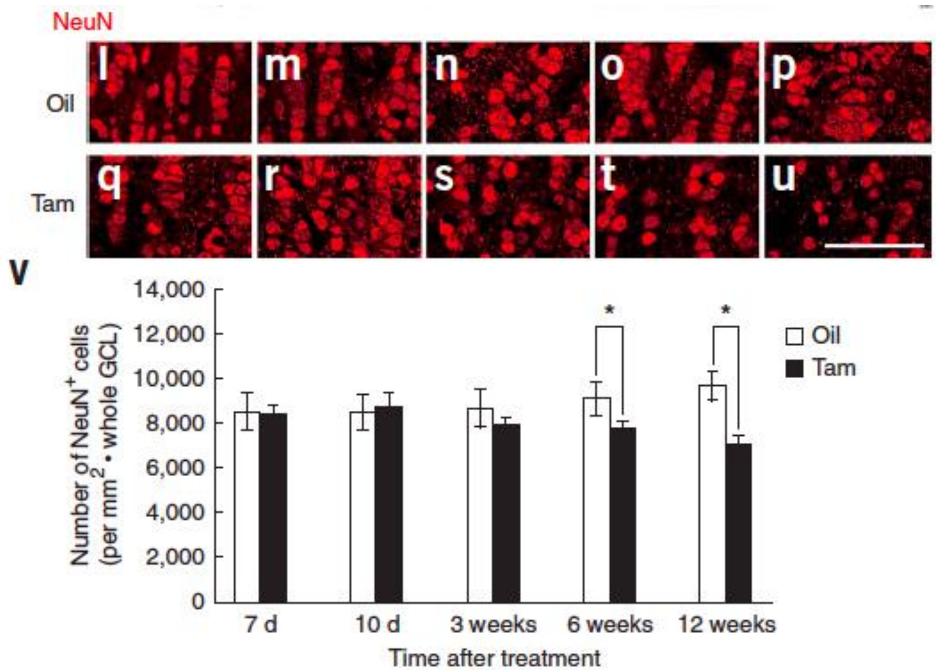
Itaru Imayoshi[1–3], Masayuki Sakamoto[1,2], Toshiyuki Ohtsuka[1,3], Keizo Takao[4–6], Tsuyoshi Miyakawa[4–6], Masahiro Yamaguchi[7], Kensaku Mori[7], Toshio Ikeda[8,9], Shigeyoshi Itohara[8] & Ryoichiro Kageyama[1,3]

Nature Neuroscience 2008

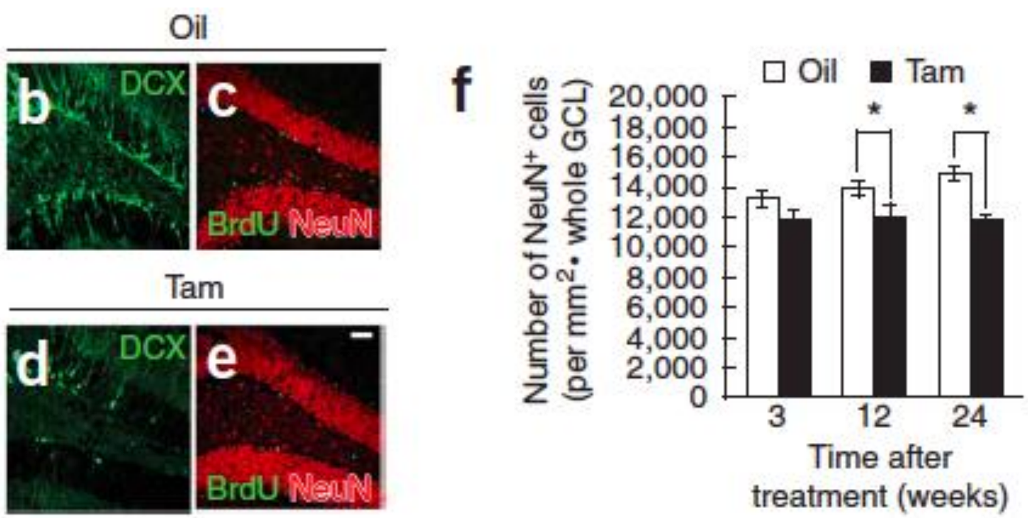


- excision of stop cassette in adult neural stem cells expressing nestin (NesCreER)
- diphteria toxin (DT-A) under neuronal lineage specific promoter (NSE)
- upon differentiation, newborn neurons get killed because of DT-A expression

Functional significance of adult neurogenesis

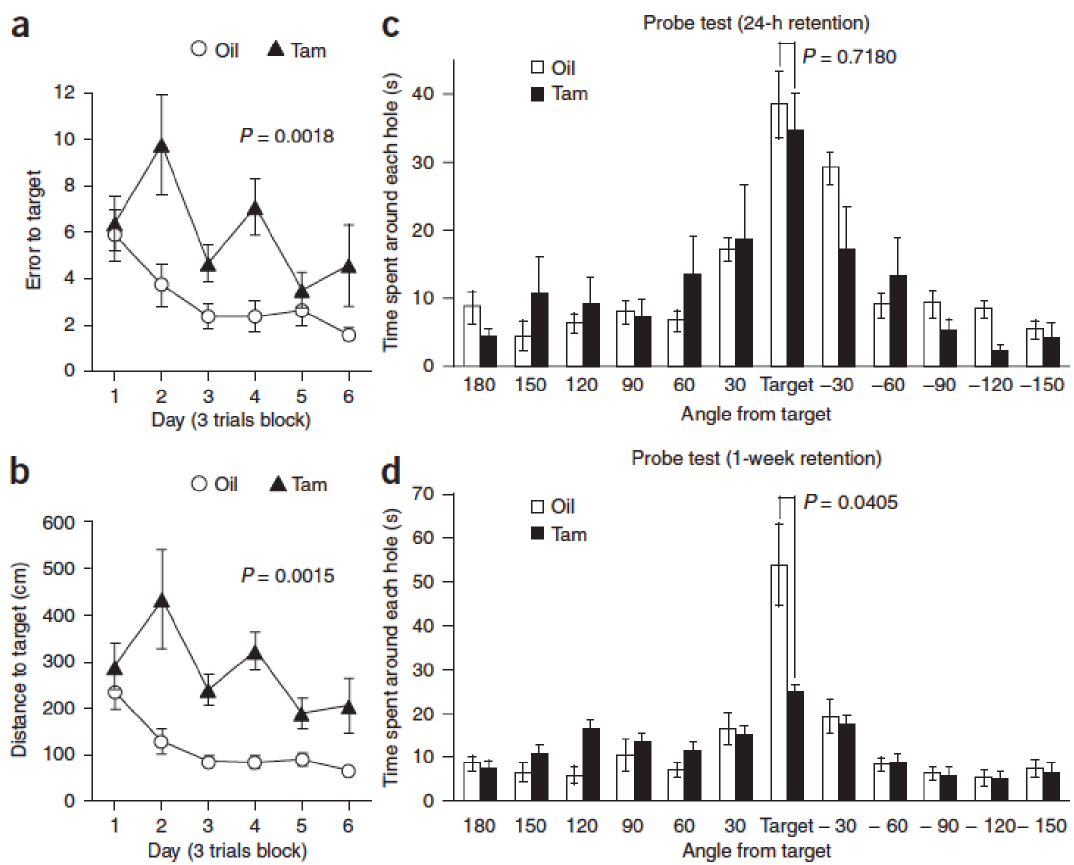


olfactory bulb



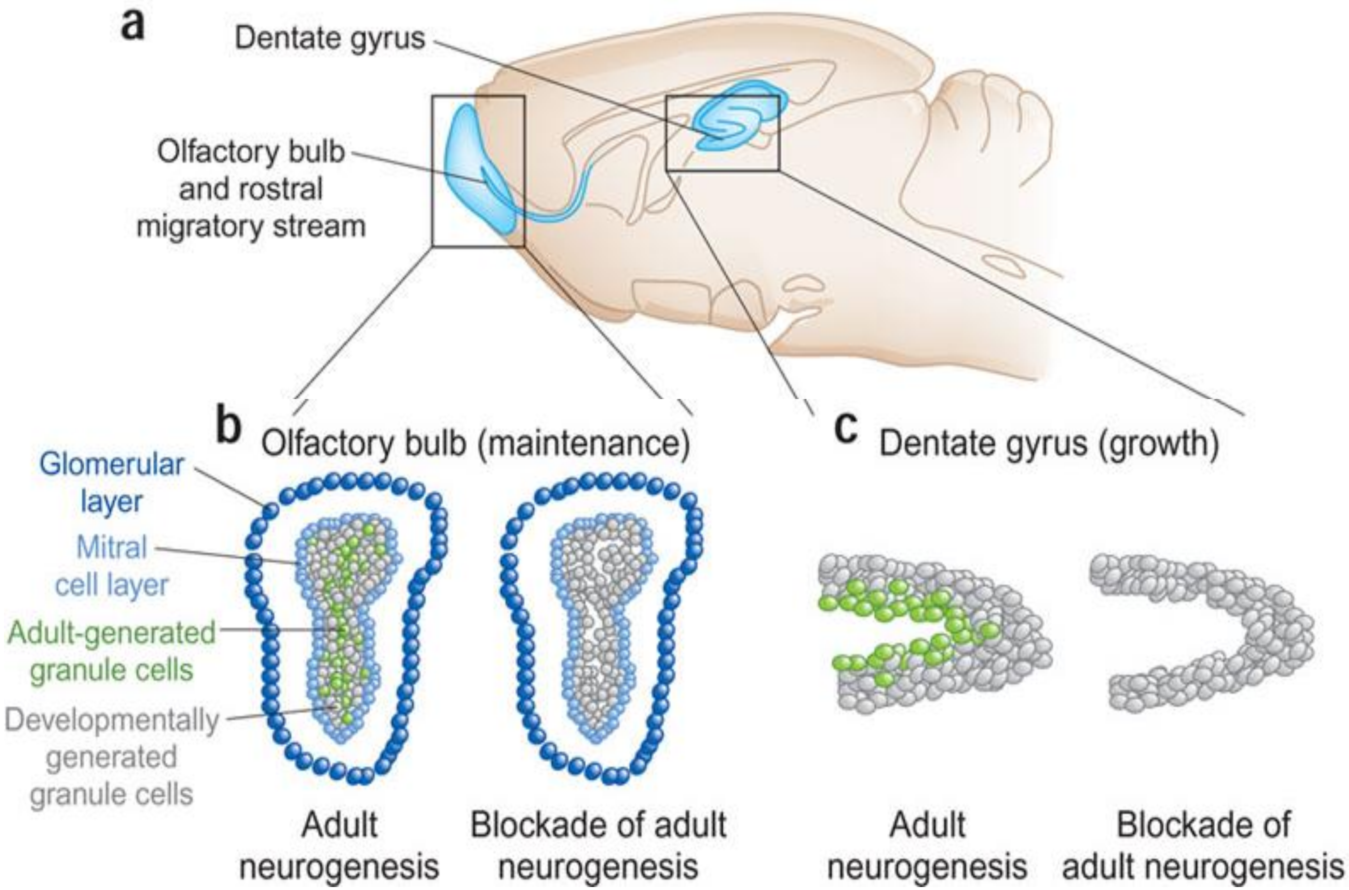
dentate gyrus

Functional significance of adult neurogenesis



Barnes Maze

Functional significance of adult neurogenesis



(Frankland and Miller / Imayoshi et al. Nature Neuroscience, 2008)

The dentate gyrus as a pattern separator

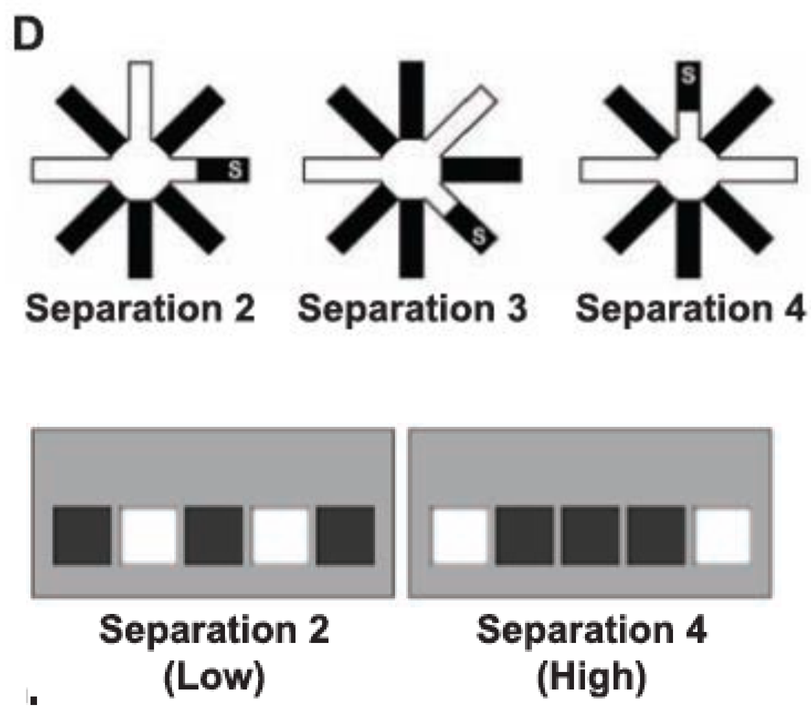
Adult neurogenesis: pattern separation

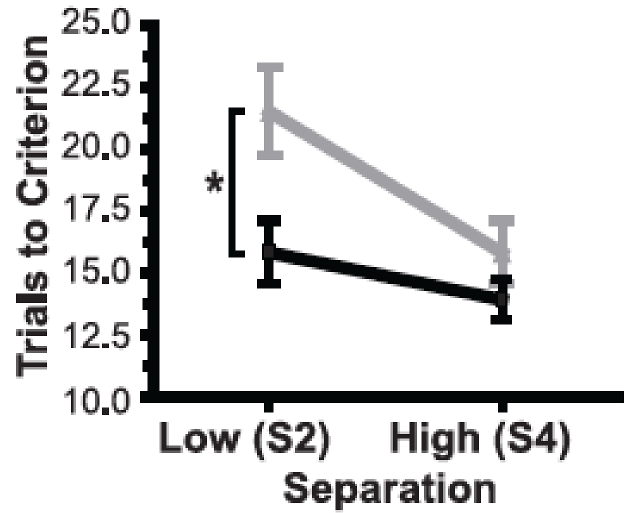
A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation

C. D. Clelland,[1,2] M. Choi,[2] C. Romberg,[3] G. D. Clemenson Jr.,[1] A. Fragniere,[2] P. Tyers,[2] S. Jessberger,[4] L. M. Saksida,[3,5] R. A. Barker,[2,6]* F. H. Gage,[1]*† T. J. Bussey[3,5]*†

Science 2009

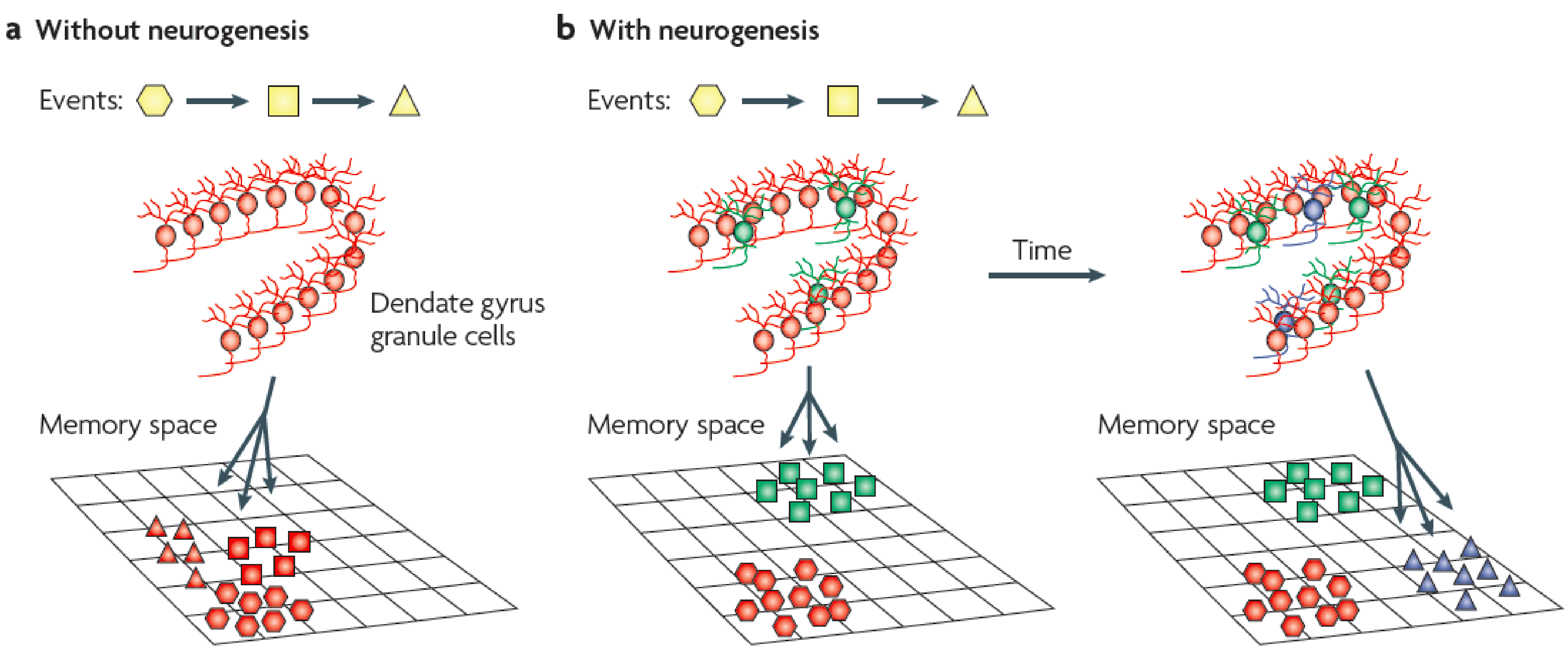
low dose irradiation, behavioral testing for spatial discrimination





Adult neurogenesis: pattern separation

newborn neurons are initially more excitable than adult neurons (lower threshold to react to stimulus)



Deng et al 2010,
Nature Reviews Neuroscience

Using neural stem cells for research & therapy

neural stem cells for diagnostics/studying degenerative diseases (patient derived iPS cells)

neural stem cells for transplantation / central nervous system repair

Patient derived iPS generated neural stem cells for diagnostics

- how well is a “neurological” disease conserved ex vivo
- most degenerative diseases occur only at older age, are aging effects gone through reprogramming?

iPS cells from Patients fibroblasts to study Schizophrenia and Alzheimer’s disease

Modelling schizophrenia using human induced pluripotent stem cells

Kristen J. Brennand[1], Anthony Simone[1]*, Jessica Jou[1]*, Chelsea Gelboin-Burkhart[1]*, Ngoc Tran[1]*, Sarah Sangar[1], Yan Li[1], Yangling Mu[1], Gong Chen[2], Diana Yu[1], Shane McCarthy[3], Jonathan Sebat[4] & Fred H. Gage[1]

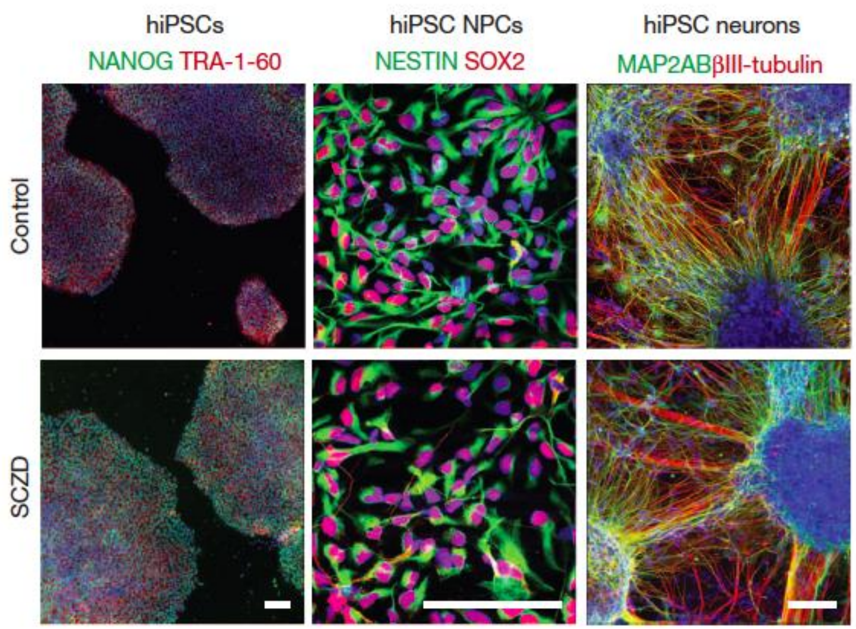
Nature. 2011

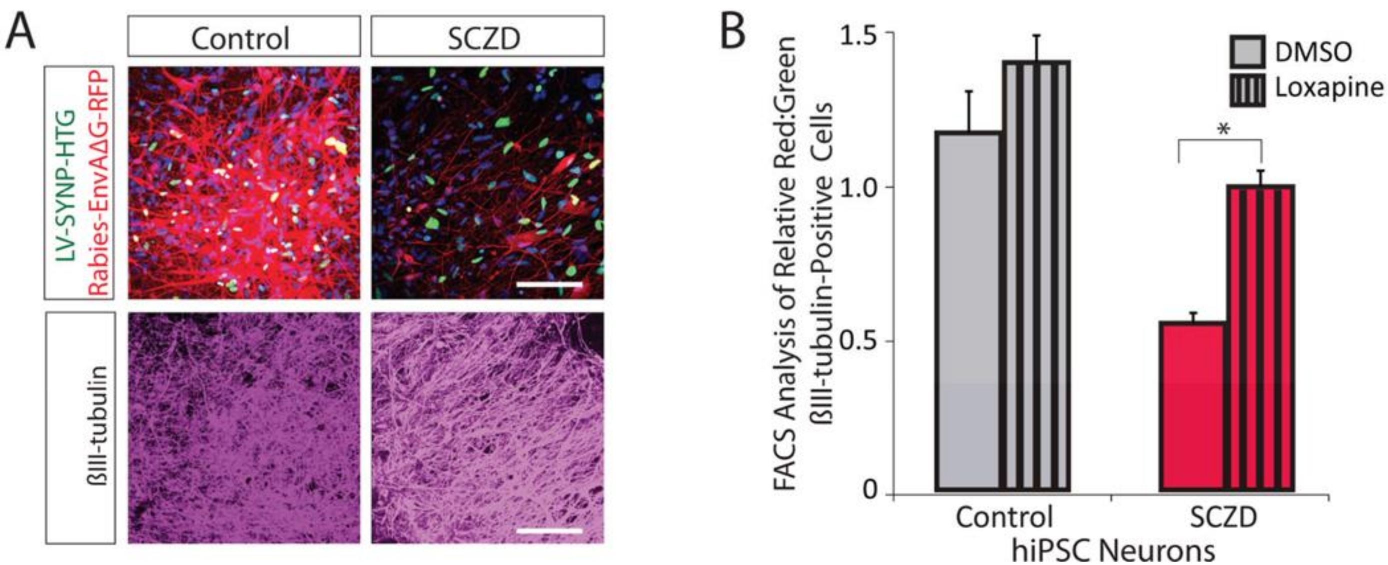
Probing sporadic and familial Alzheimer’s disease using induced pluripotent stem cells

Mason A. Israel[1,2], Shauna H. Yuan[1,3], Cedric Bardy[4], Sol M. Reyna[1,2], Yangling Mu[4], Cheryl Herrera[1], Michael P. Hefferan[5], Sebastiaan Van Gorp[6], Kristopher L. Nazor[7], Francesca S. Boscolo[8], Christian T. Carson[9], Louise C. Laurent[8], Martin Marsala[5,10], Fred H. Gage[4], Anne M. Remes[11], Edward H. Koo[3] & Lawrence S. B. Goldstein[1,3]

Nature. 2012

Patient derived NSCs and neurons: Schizophrenia



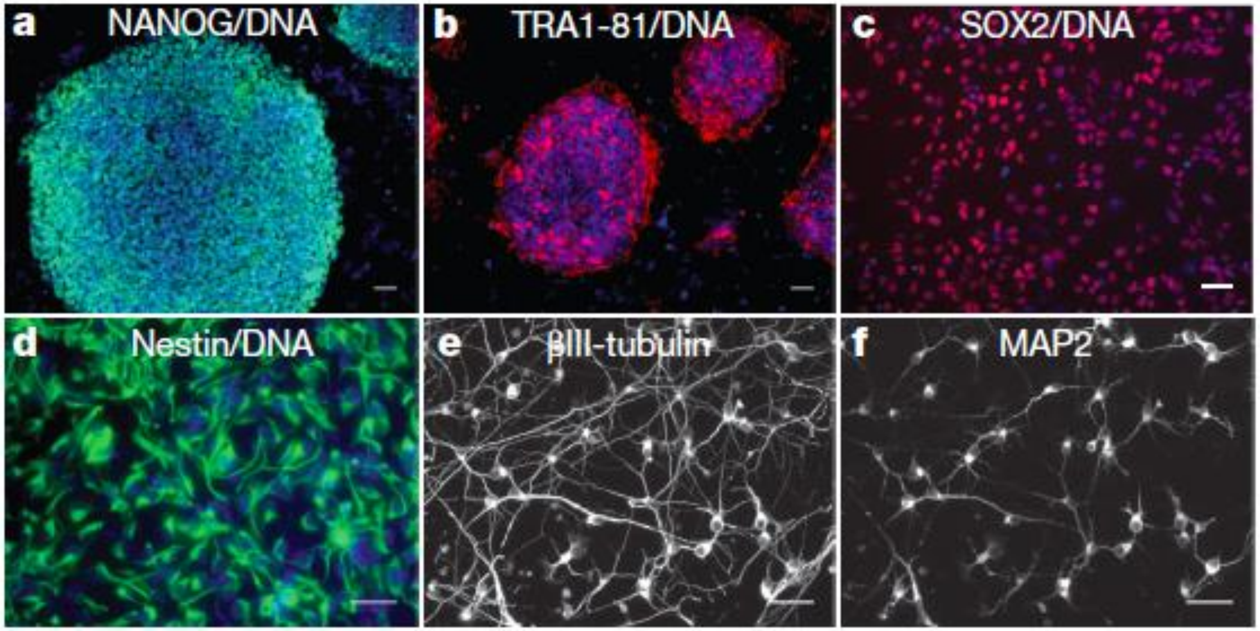


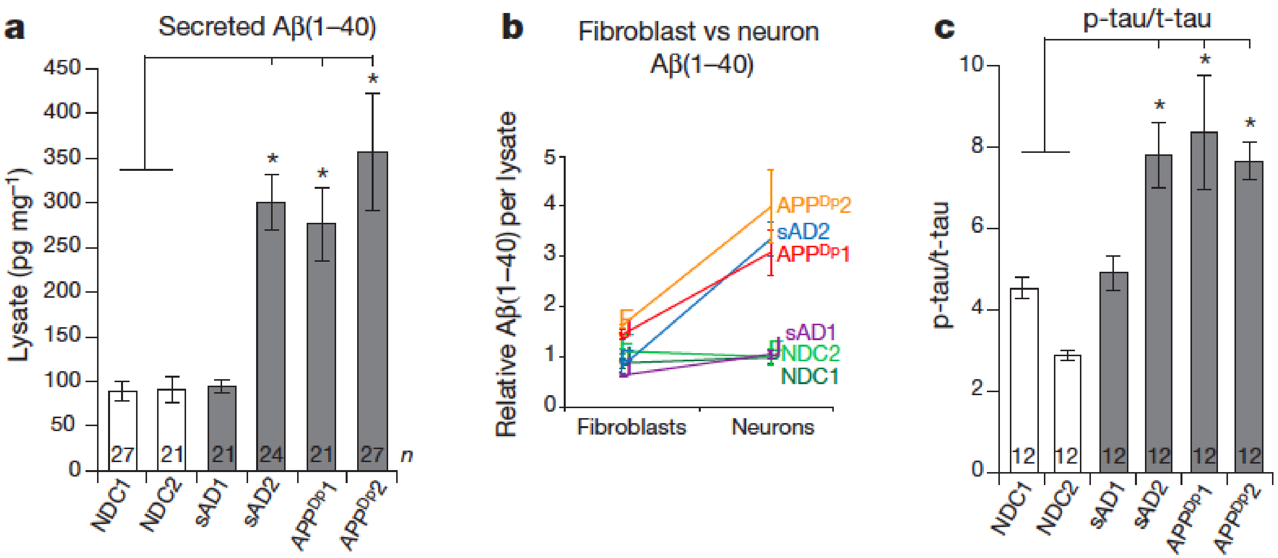
Brennand et al. 2011

Patient derived NSCs and neurons: Alzheimer's disease

Alzheimer's disease:

- most common form of dementia
- small % clearly genetic
- 2 "hallmarks":
  Aβ accumulation
  (amyloid plaques)
  hyperphos-tau
  (neurofibr. tangles)

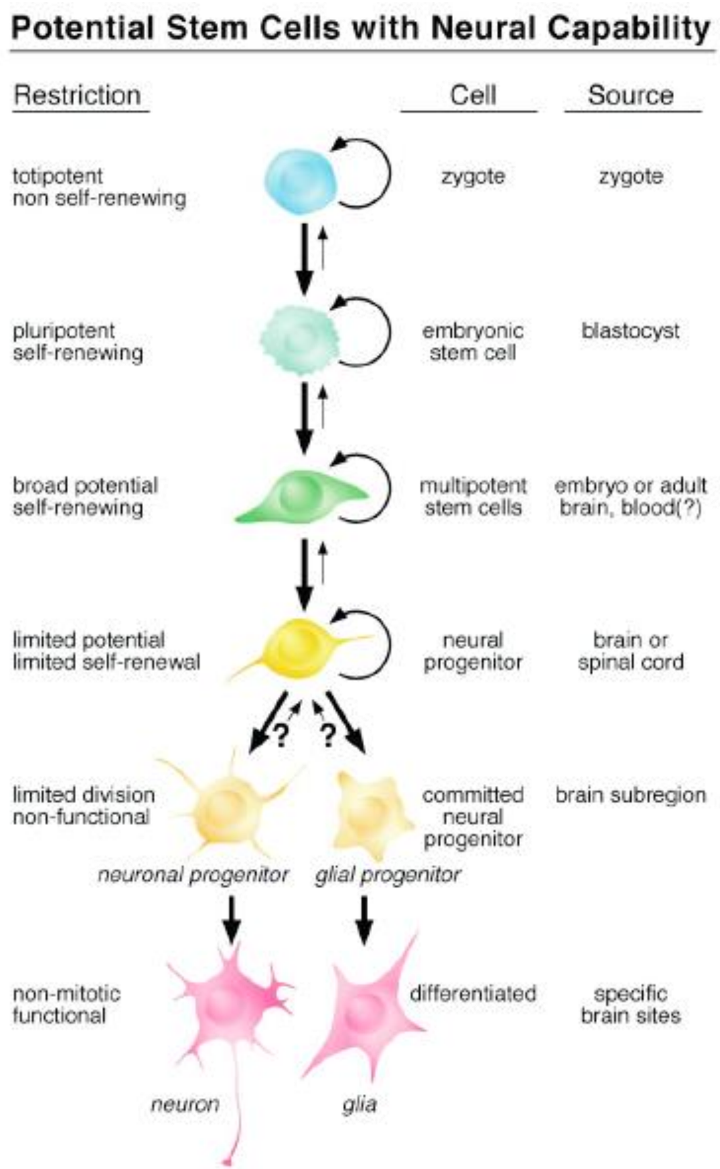




"hallmark" AD proteins: Aβ and p-tau

increased in neurons via iPS generated neurons from AD patients

Neural stem cell transplantations: source?



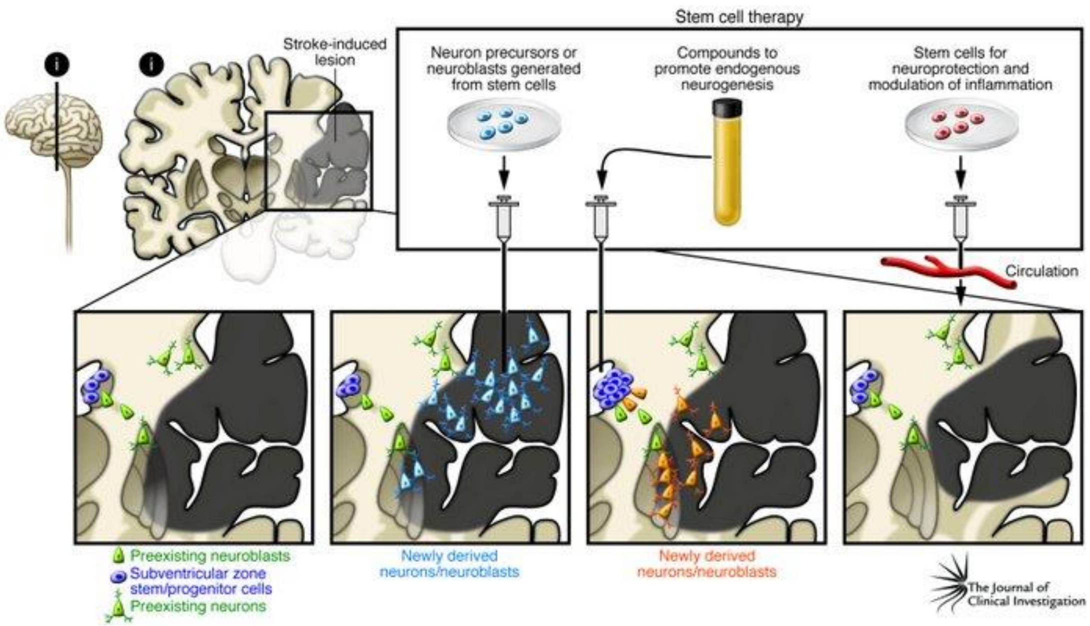
Gage, Science 2000

Safety
(uncontrolled growth, tumor formation)

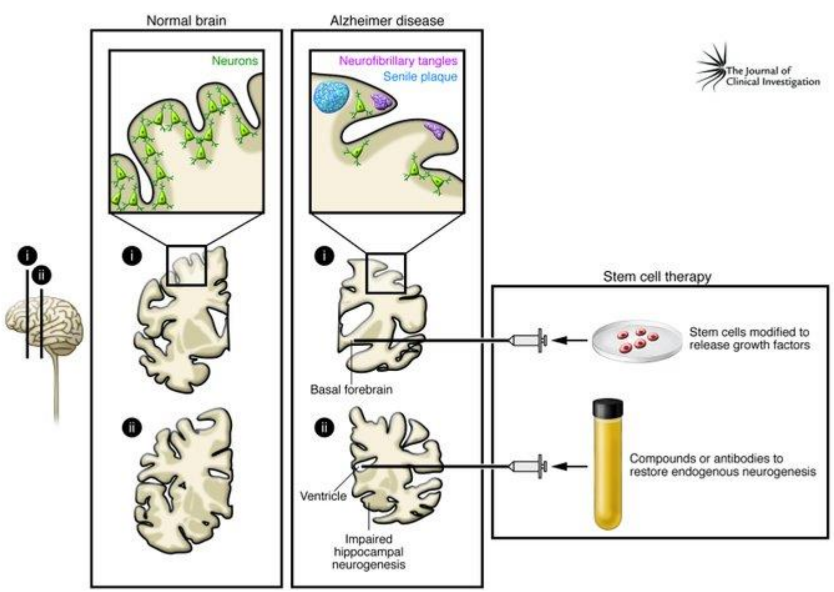
Ethical concern
(e.g. embryonic source,
or fetal brains from abortions)

Availability

Neural stem cell transplantations: several diseases to target



Stroke



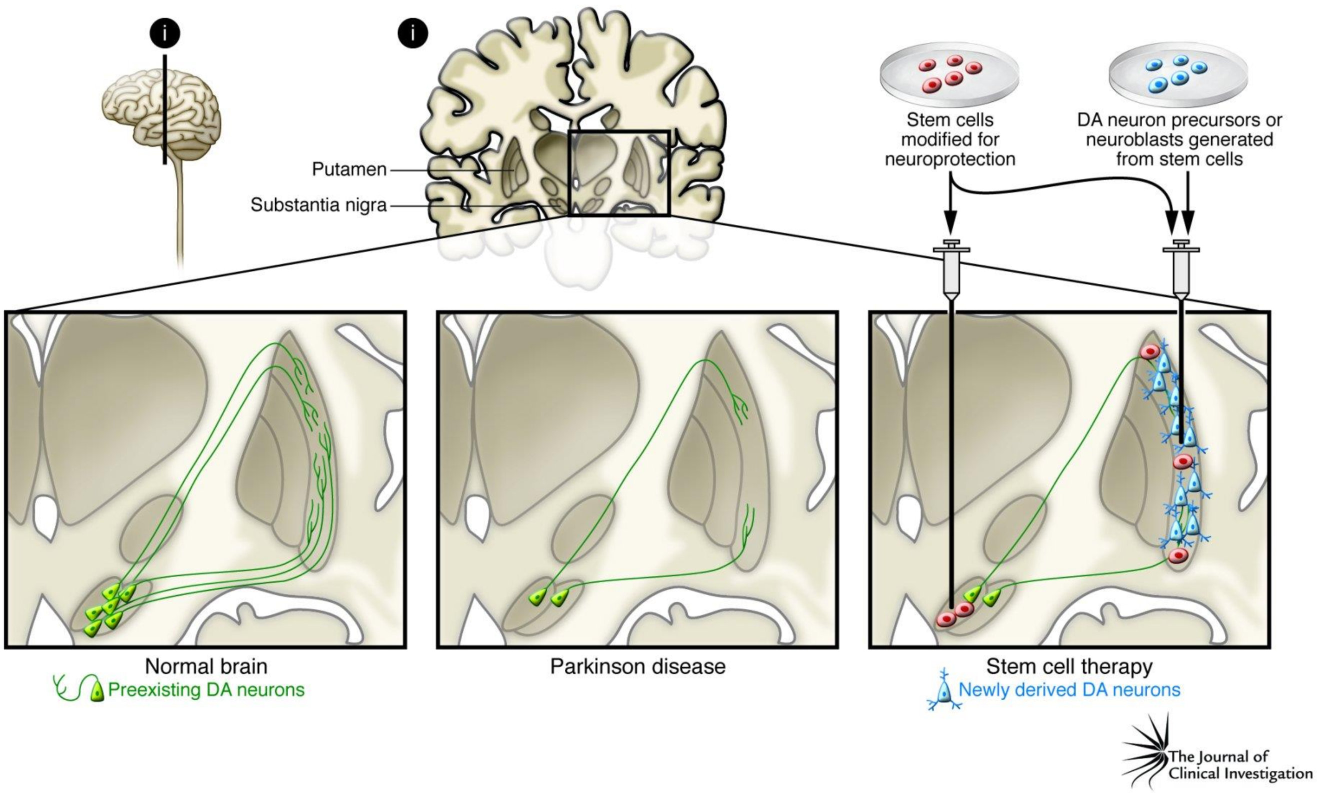
Alzheimer's disease

Lindvall & Kokaia, 2010

Neural stem cell transplantations: Parkinson's disease

Parkinson's disease:
degeneration of the dopaminergic neurons in the substantia nigra

Symptoms: impaired movement (rigidity, hypokinesia, tremor), dementia



Lindvall & Kokaia, 2010

Cell transplantations into the brain: pioneering work

clinical studies started 1987

transplantation of human fetal mesencephalic tissue (rich in dopaminergic neurons) to the striatum in PD patients

summarized outcome:

1) the grafted dopaminergic neurons survived and formed connections in the patient's brain

1) the patient's brain could integrate and use the grafted neurons

2) the grafts did induce measurable clinical improvement

Safety? ethical concern? availability?

Neural stem cell transplantations

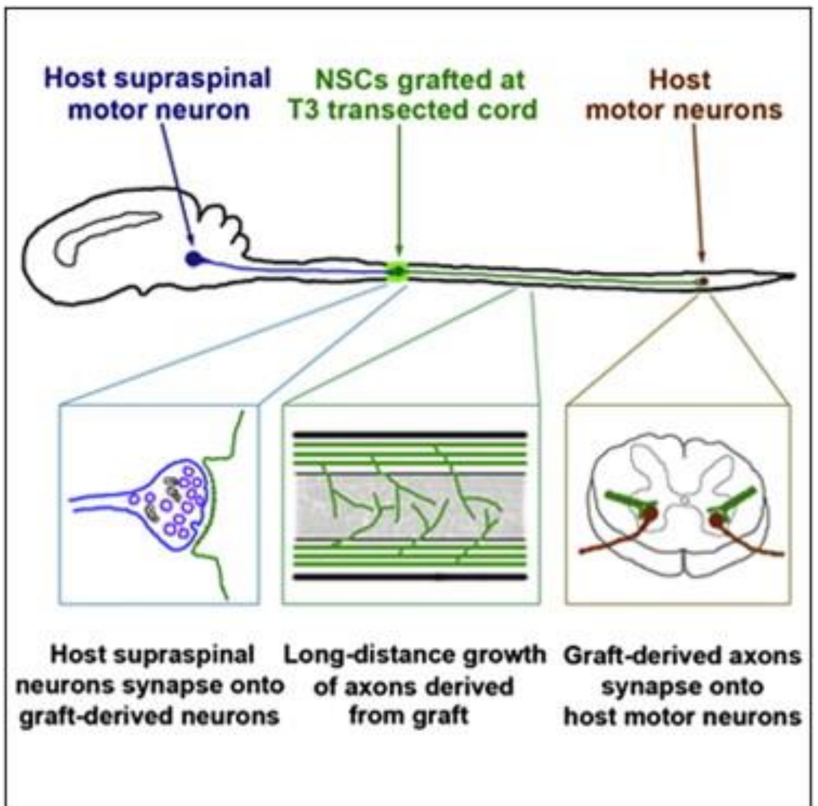
verification in animal models (mostly higher mammals such as rhesus monkey)

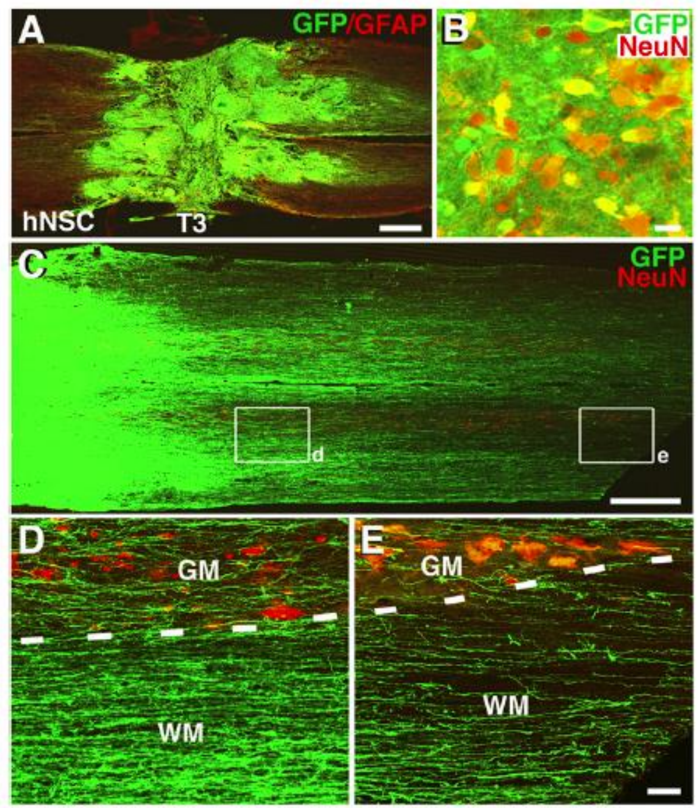
Neural stem cell transplantations

Cell Sept. 2012

Long-Distance Growth and Connectivity of Neural Stem Cells after Severe Spinal Cord Injury

Paul Lu,[1,3,*] Yaozhi Wang,[1] Lori Graham,[1] Karla McHale,[1] Mingyong Gao,[1] Di Wu,[1] John Brock,[1] Armin Blesch,[1,5] Ephron S. Rosenzweig,[1] Leif A. Havton,[4] Binhai Zheng,[1] James M. Conner,[1] Martin Marsala,[2] and Mark H. Tuszynski[1,3,*]





Next lecture on 3.10.25
Tools in stem cell biology