Developmental Neurobiology

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UNIT 4

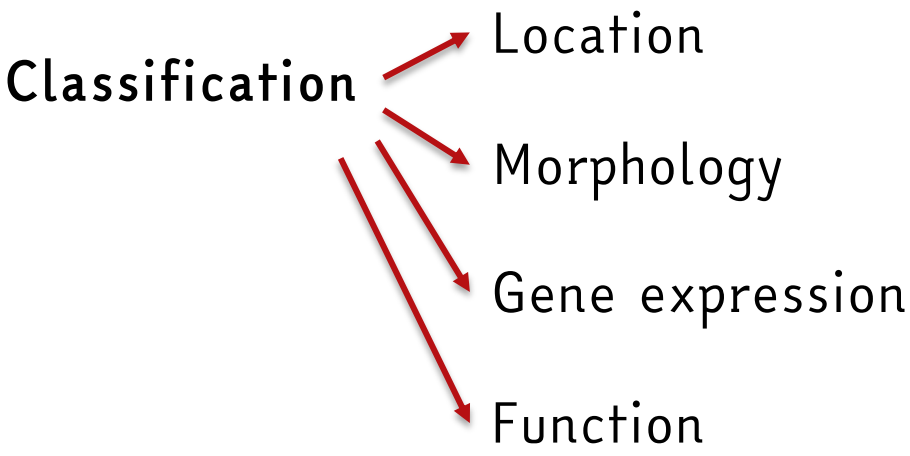
Neuro-gliogenesis

Neural Progenitor Cells

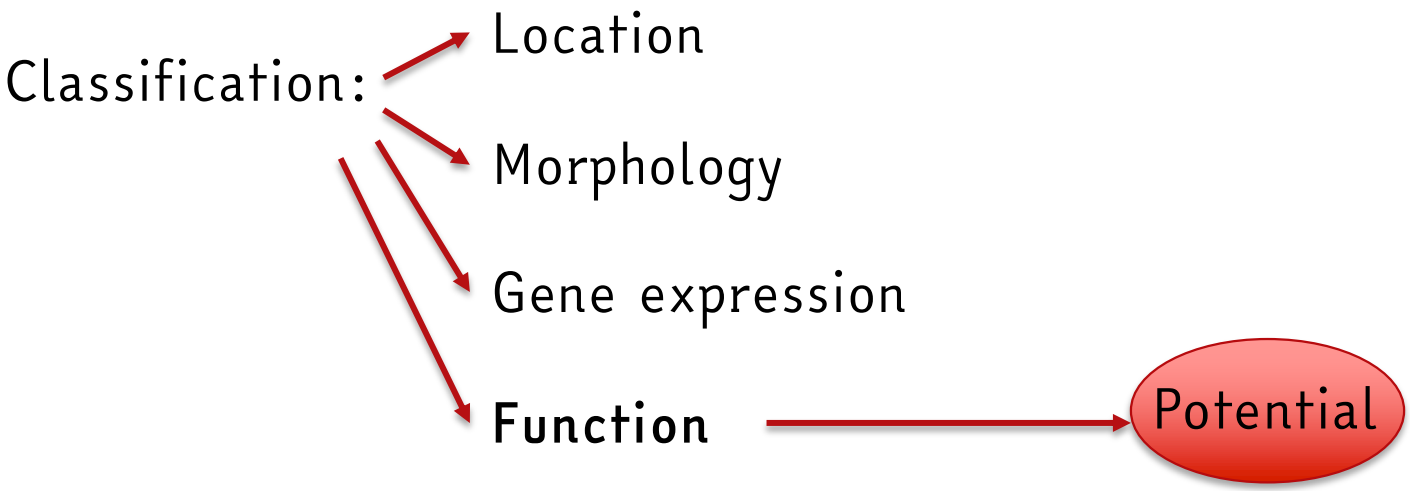
Neural Progenitor Cells (NPCs)

- Are the progenitors of the CNS
- Originate all the glial (macroglia) and neuronal cell types of CNS
- Do not generate non-neural cells of the CNS
- Are found in the embryonic, neonatal and adult CNS
- Can be generated *in vitro* by ESCs or iPSCs

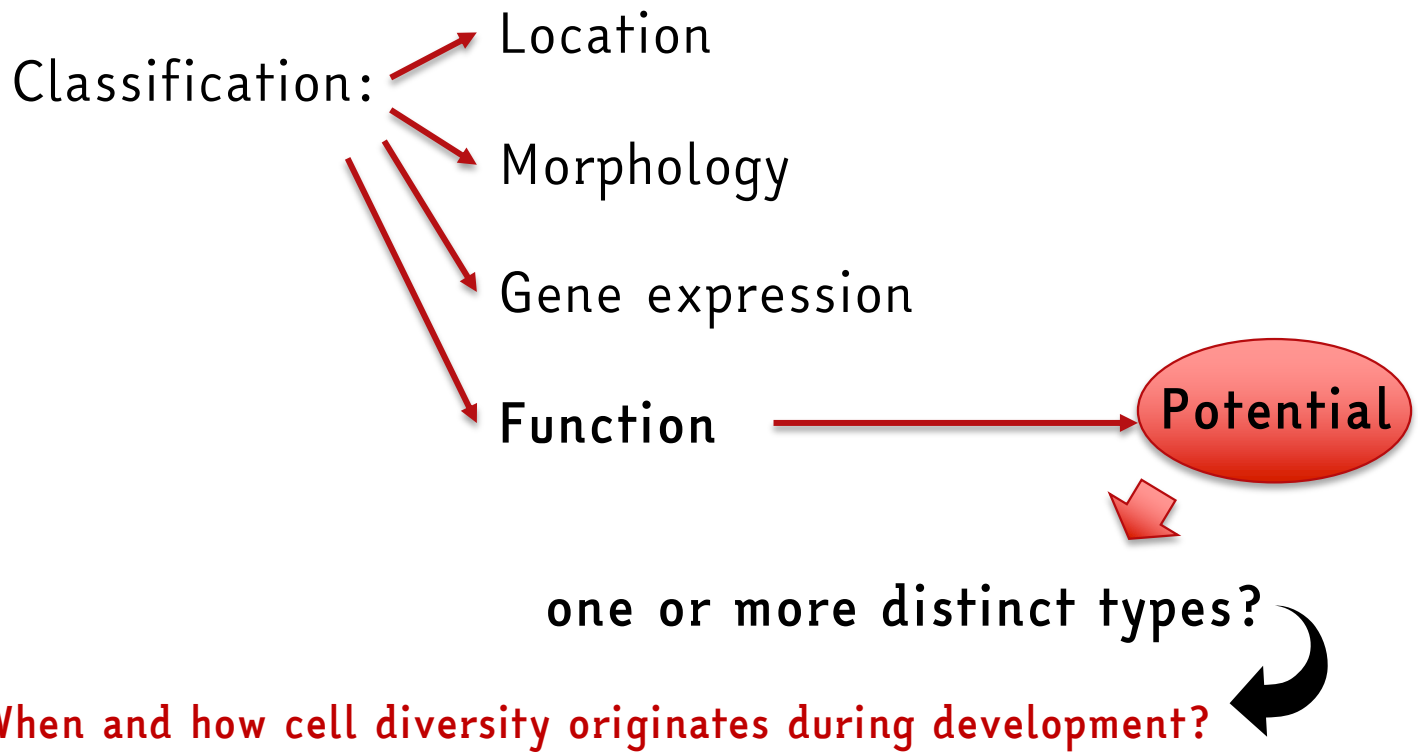
Neural Progenitor Cells (NPCs)



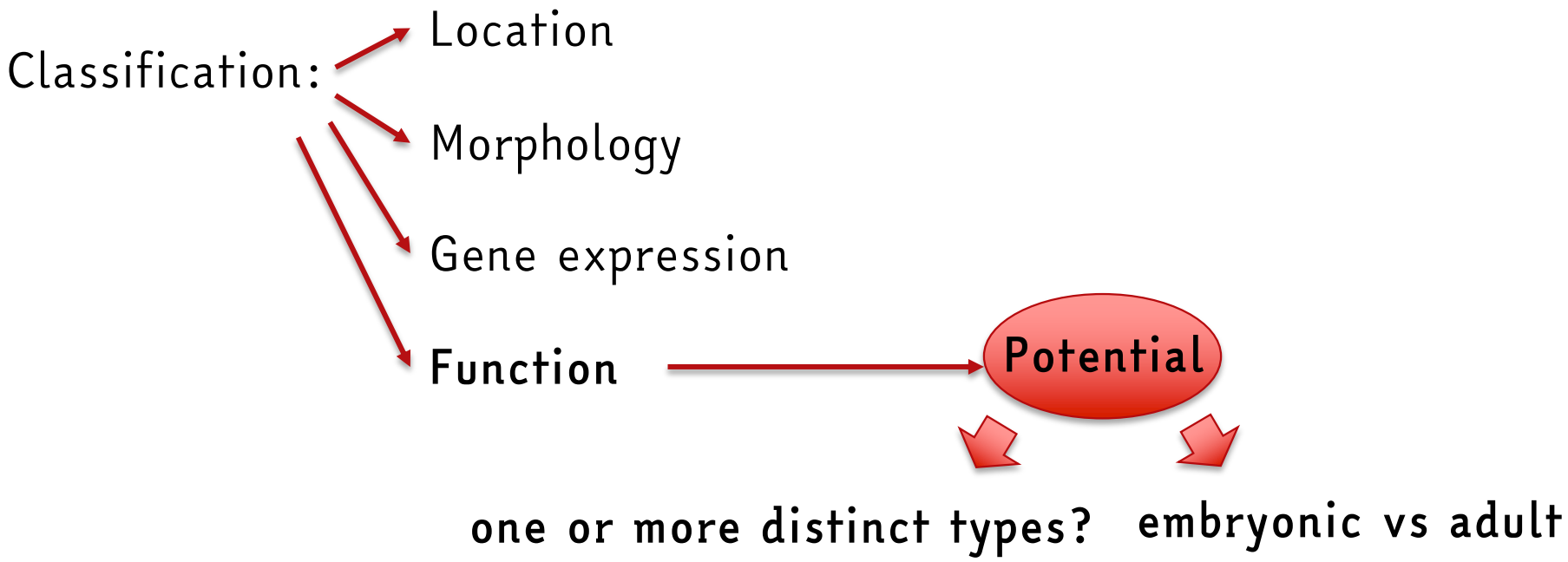
Neural Progenitor Cells (NPCs)



Neural Progenitor Cells (NPCs)

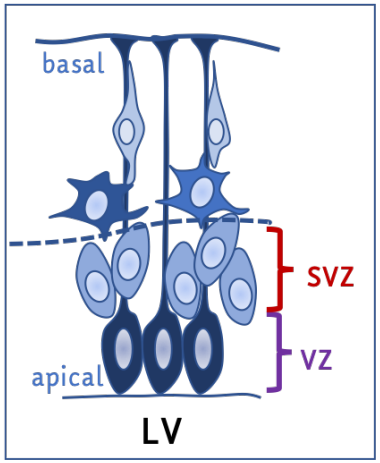


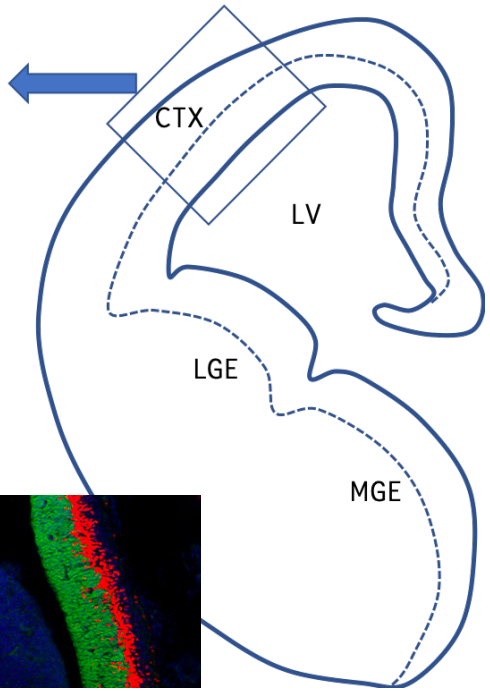
Neural Progenitor Cells (NPCs)

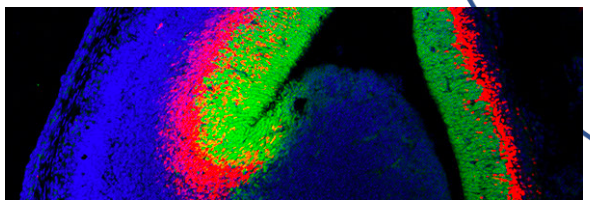


Location of embryonic neural proliferative zones

In the developing forebrain the VZ and SVZ represent the neural proliferative zones





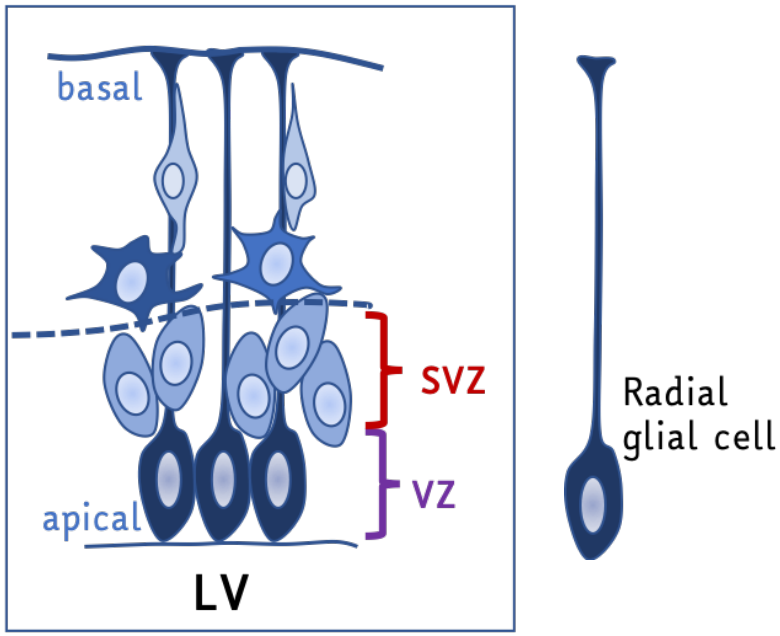


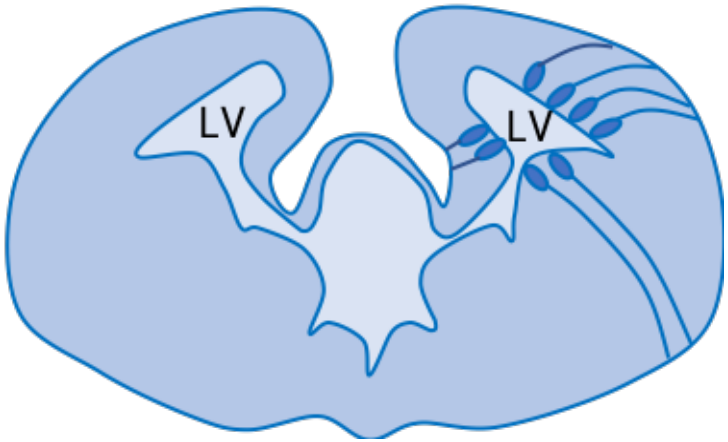
The VZ (ventricular zone) → adjacent to the ventricle, is the primary proliferative zone (the first to appear during development)

The SVZ (subventricular zone) → is superficial to the VZ - It is the secondary proliferative zone (appears later in development)

In the cerebral cortex it can be subdivided in **outer SVZ** and **inner SVZ**

Radial glial cells





Radial glial cells

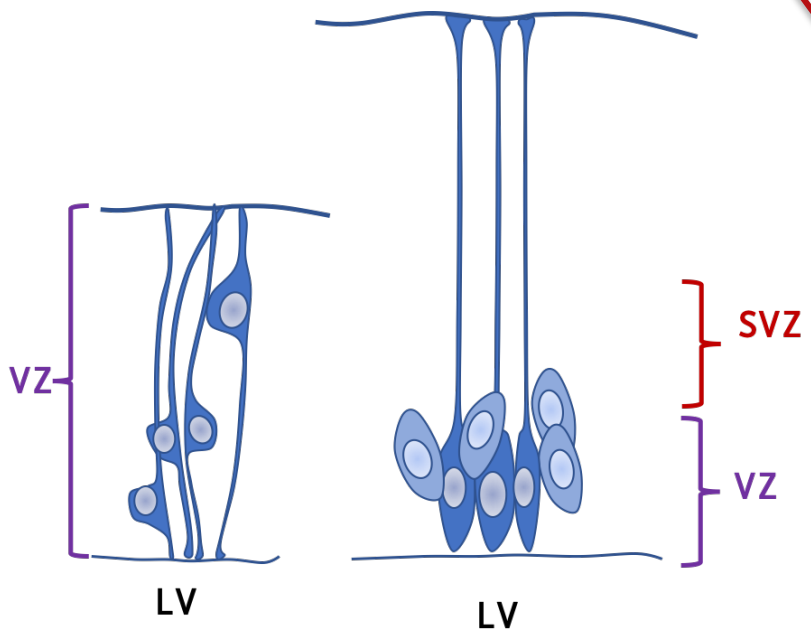
Radial glial cells derive from neuroepithelial cells

(starts at E9-E10 in mice
human GW 5-6)

Neuroepithelial cells → **Radial Glial Cells**

typical epithelial features
highly polarized along their apical–basal axis

Self-renewing symmetric divisions



residual neuroepithelial
+
astroglial features
(down-regulate tight junctions)

Table 1 | **Comparison of the properties of neuroepithelial and radial glial cells**

Property	Neuroepithelial cells	Radial glial cells
Interkinetic nuclear migration	Apical–basal	Apical–basal to the boundary of the ventricular or subventricular zone
Apical surface	Present	Present
Apical–basal polarity	Present	Present, but downregulated
Tight junctions	Present (early stages)	Absent
Adherens junctions	Present	Present
Basal lamina contact	Present	Present
Nestin expression	Present	Present
Astroglial markers	Absent	Present
Tis21 expression*	Confined to the neurogenic subpopulation	Present in the neurogenic subpopulation
Neurogenesis	First phase	Subsequent phases

*The antiproliferative gene *Tis21* is a molecular marker that is selectively expressed in virtually all neuroepithelial cells that are about to undergo a neurogenic division, but not in proliferating neuroepithelial cells[74].

Antigen/feature	Neuroepithelial cells	Radial Glia	Astrocytes
Nestin	+	+	- (reinduced in reactive astrocytes)
RC2	+	+	- (reinduced in reactive astrocytes)
RC1	+	+	+
GFAP	-	+ (absent in rodents)	+
GLAST	-	+	+
BLBP	-	+	+
TNC	-	+	+
Glycogen granules	-	+	+

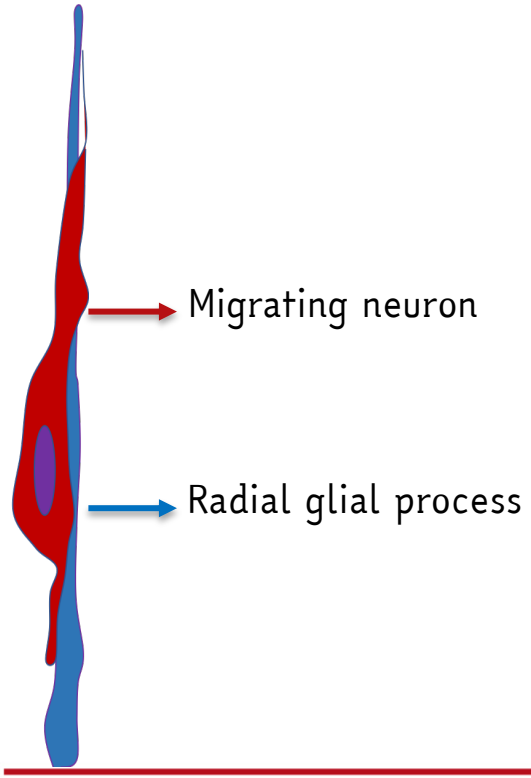
Malatesta et al., 2003

Glast = glutamate aspartate transporter
BLBP = brain lipid-binding protein (FABP7)
TNC = tenascin C
RC2 = post-translationally modified isoform of Nestin (Park et al., 2009)

The radial glial cells

Radial Glia → term introduced by Pasko Rakic (1971)

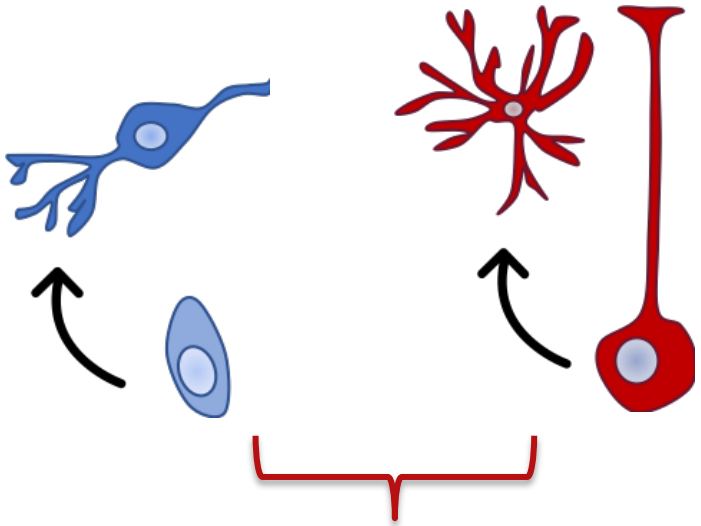
Cerebral cortex development



Function:

architectural framework for neuronal (radial)

The concept of dualism between NEURONAL and GLIAL lineages



Co-existence of **neuroblasts** and **spongioblasts* based on soma position**

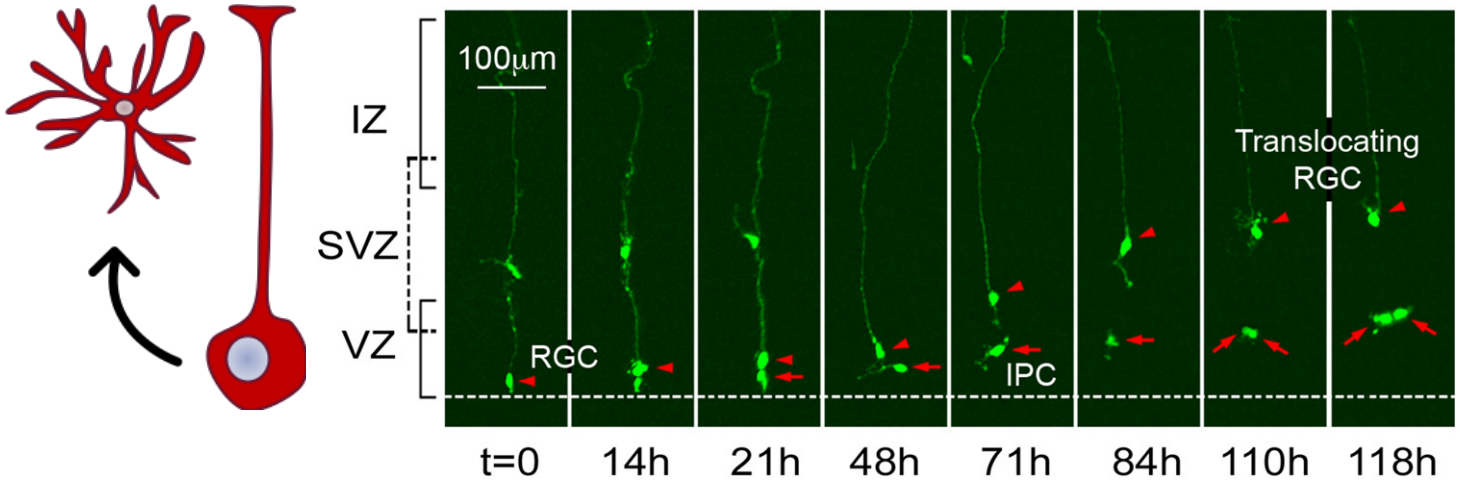
→ but soma position corresponds to different phases of cell cycle (M apical; S basal)

Spongioblasts* as progenitors of glial cells:

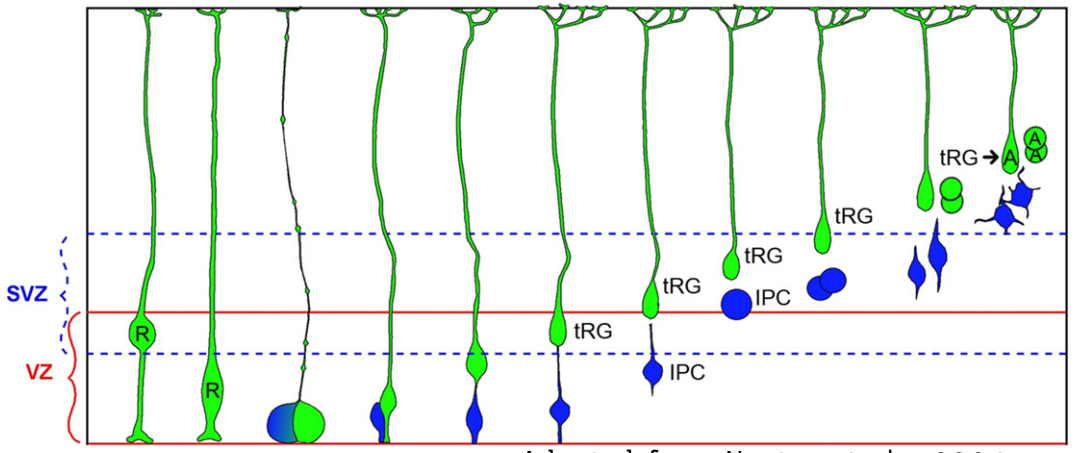
-Morphological similarity (Gogli 1885; His 1889)
-GFAP expression (Levitt and Rakic 1980)

→Lineage-tracing studies with lipophilic dye on ferret cortex, showed transitional forms between radial glia and astrocytes at the end of the neurogenesis (Voigt, 1989) supporting the existence of distinct progenitors for neurons and astrocytes

*how radial glia was named by Camillo Golgi in 1885 following observation of radial processes in the foetal spinal cord



Organotypic slice culture from embryonic rat E18



Adapted from Noctor et al., 2004

tRG = translocating radial cell
A = astrocytes

tRG other fate?

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Development 127, 5253-5263 (2000)
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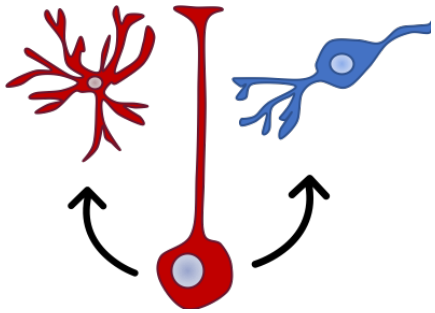

Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage

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Accepted 3 October; published on WWW 14 November 2000

Head of LMU Department of Physiological Genomics
Helmholz Center Munich,
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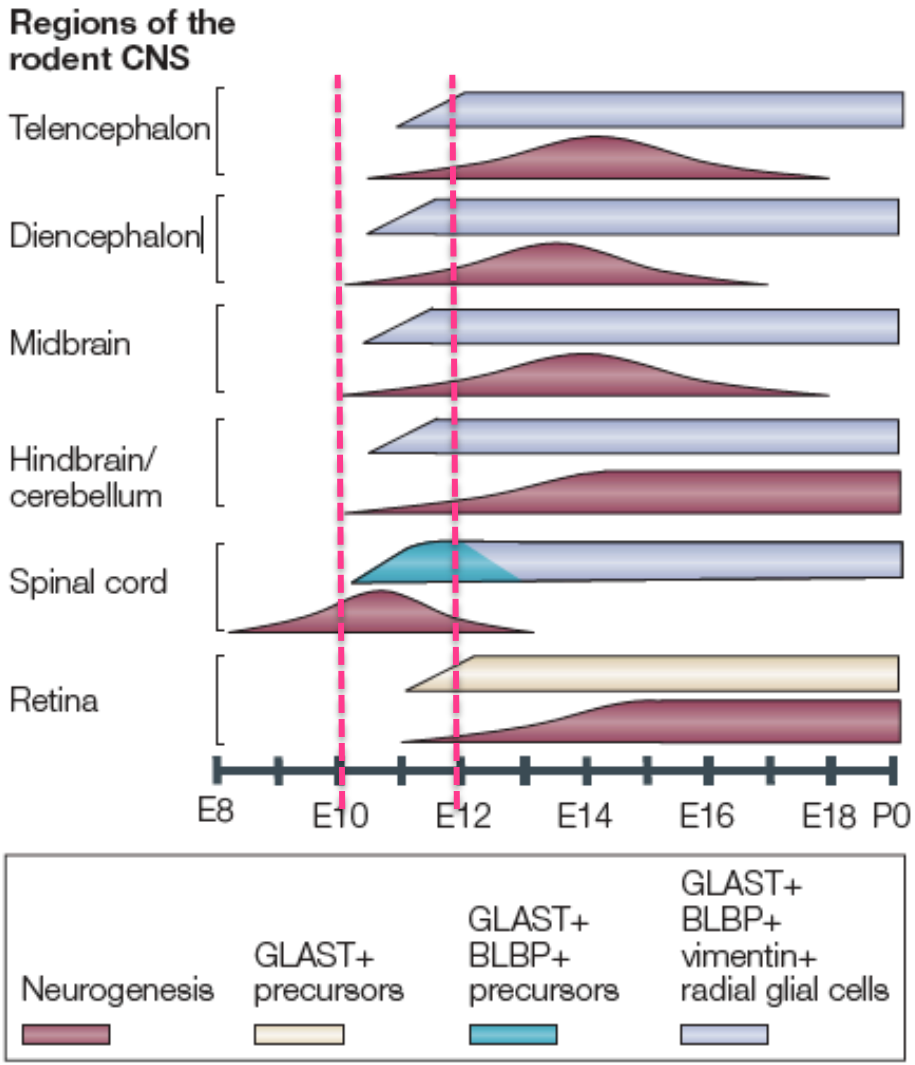
SUMMARY

The developing central nervous system of vertebrates contains an abundant cell type designated radial glial cells. These cells are known as guiding cables for migrating neurons, while their role as precursor cells is less clear. Since radial glial cells express a variety of astroglial characteristics and differentiate as astrocytes after completing their guidance function, they have been considered as part of the glial lineage. Using fluorescence-activated cell sorting, we show here that radial glial cells also are neuronal precursors and only later, after neurogenesis, do they shift towards an exclusive generation of astrocytes. These results thus demonstrate a novel function for radial glial cells, namely their ability to generate two major cell types found in the nervous system, neurons and astrocytes.

Key words: Cerebral cortex, Radial glial cells, Neuronal precursors, Glial precursors, Glutamate astrocyte-specific transporter (GLAST), GFP, Human GFAP-promoter, Clonal analysis



In the brain progressive waves of **neurogenesis** begin at about E 9–10 in mice

Gliogenesis follows neurogenesis
- the two processes partly opverlap –

Observation from M. Gotz lab showed that at the peak of neurogenesis virtually all **proliferating cells were RGCs**

Does it prove that RGCs are neurogenic?

From Malatesta et al., Development 2000

Experimental paradigm

to fluorescently label radial glial cells and isolate them by FACS

1 Transgenic mouse line (hGFAP-GFP)

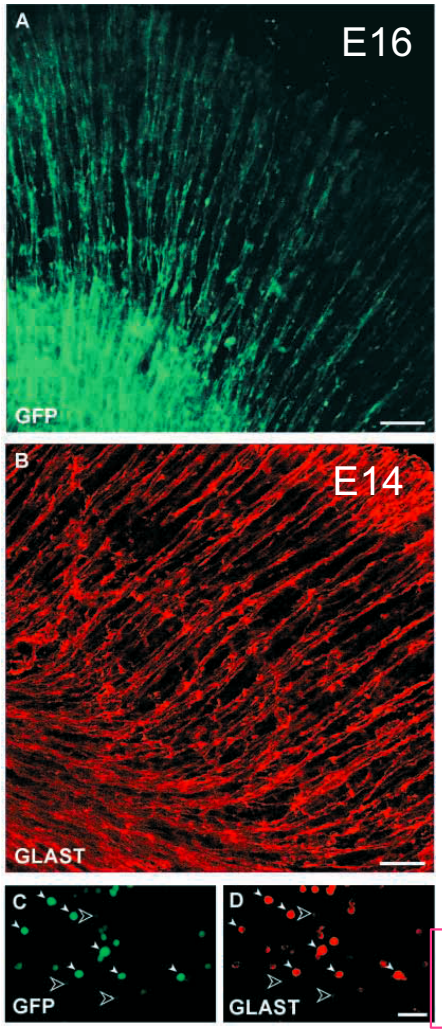
2 Labelling precursor cells with long radial processes with fluorescent dyes from the pial surface

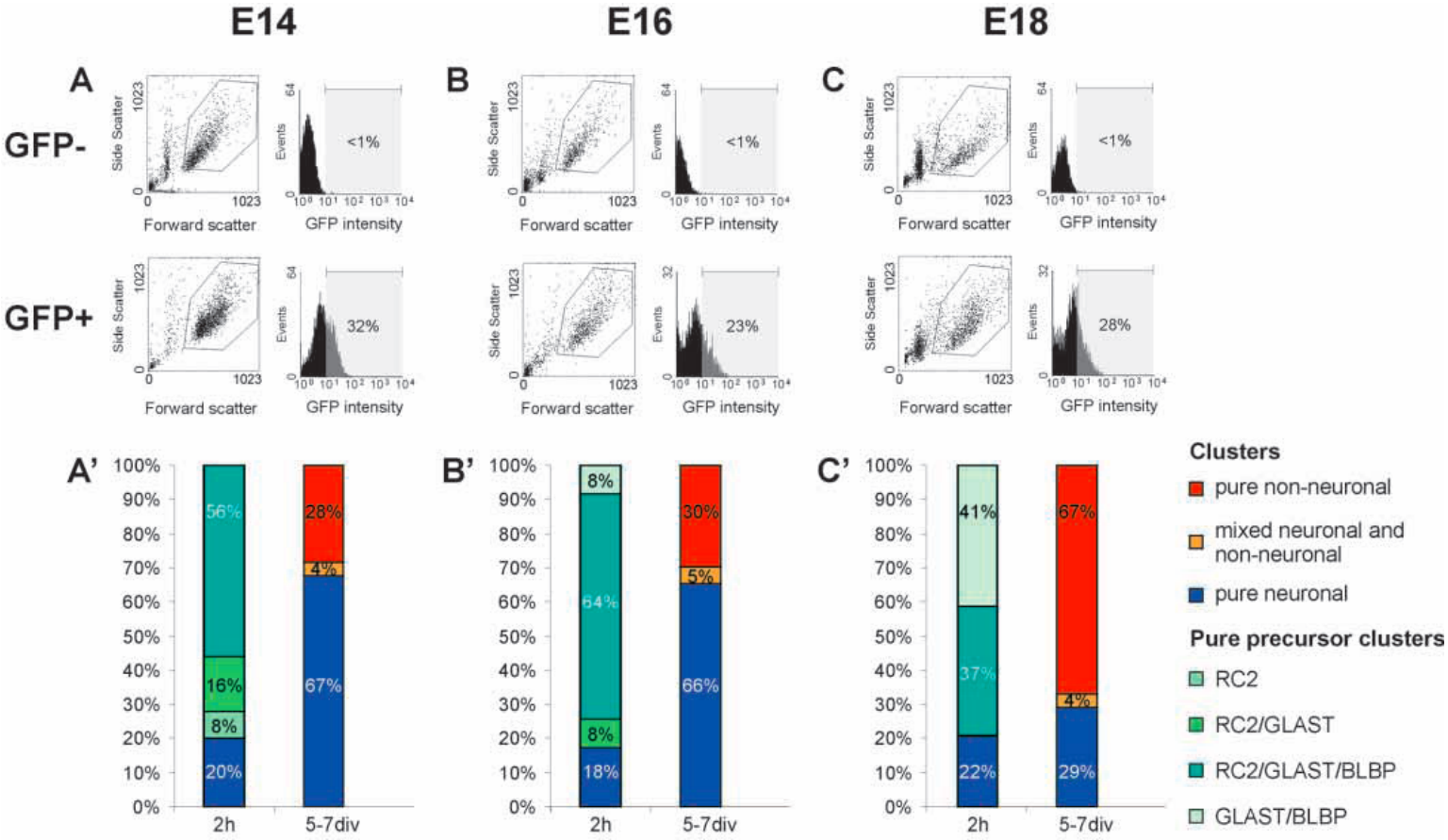
Sorted cells plated at low density on a feeder layer of cerebral cortex cells derived from rat (same age –same region) - cultured in vitro for 7 days (7div)

Progeny determined by the use of cell-type specific antibodies:

- anti-beta-tubulin-III → neurons
- anti-GFAP → astrocytes
- anti-O4→ oligodendrocyte precursors

1 hGFAP-GFP transgenic mice





GFP-immunostained cells from acutely dissociated E14 cortex double stained for GLAST

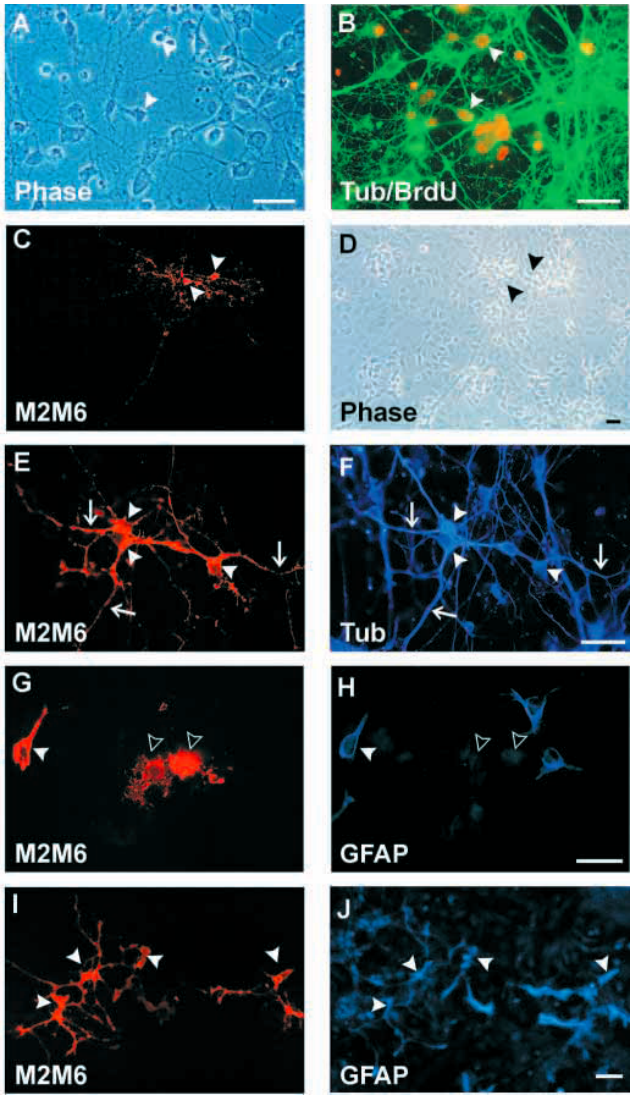
Examples of the progeny of hGFAP-GFP- and GLASTpositive precursor cells isolated by FACS after 5-7 div

1st evidence that cells of the glial lineage can generate neurons!!!

See also studies on adult neurogenesis (Doetsch et al., 1999)

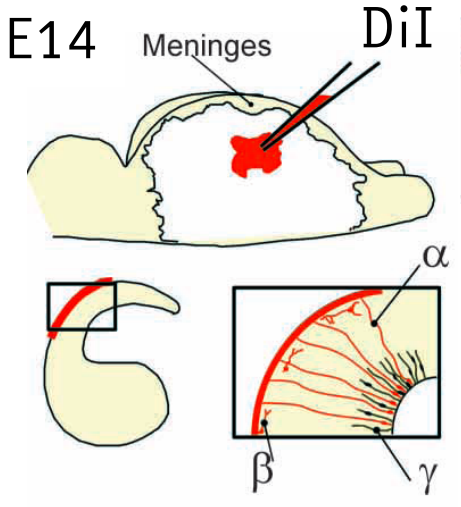
Cells were cultured on a rat cortex feeder layer of the corresponding age

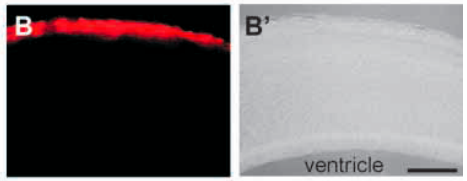
M2M6 = mouse-specific antibody



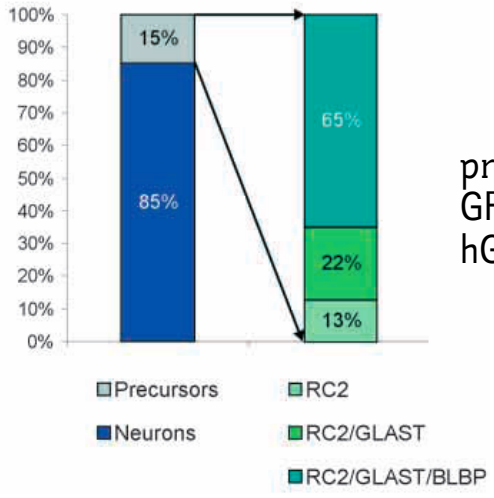
Cluster=clone derived from a single precursor cell

2 Fluorescent tracing of cells from the pial surface

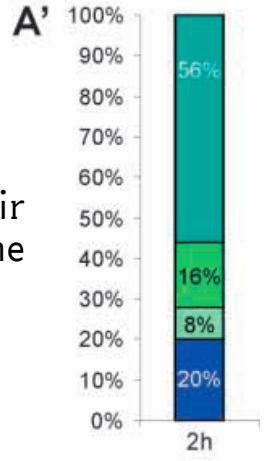




precursors sorted by their morphology via pial tracing

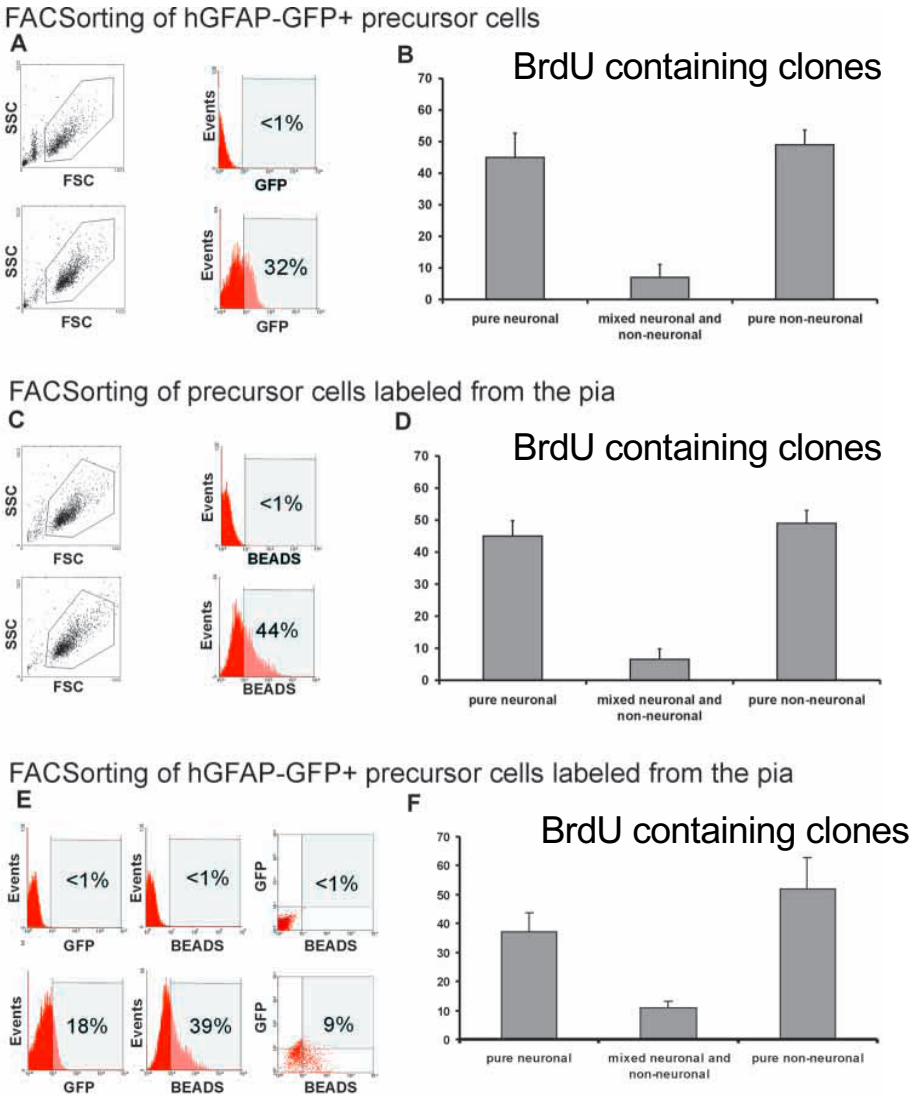


precursors sorted by their GFP-content driven by the hGFAP promoter



from E14 cortex

They **examined exclusively clones that incorporated BrdU during the time in vitro…**



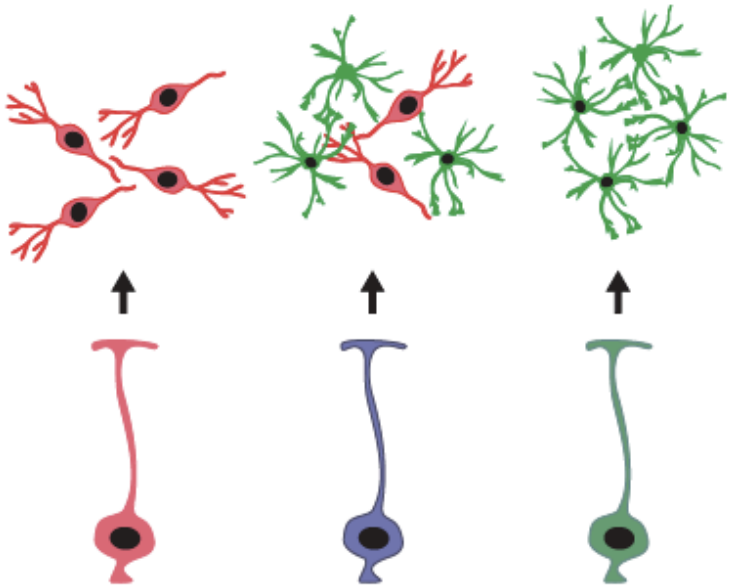


Fig. 2. Schematic summarizing the results of clonal analysis of single radial glial cells isolated by FACS as described in our *Development* paper in 2000 (Malatesta et al., 2000). Clones generated by a single cell were either composed of only neurons (red), of only glial cells (green) or of both glia and neurons. These results suggested for the first time that radial glial cells are a heterogeneous population comprising neuronal progenitors (red), glial progenitors (green) and neural stem cells (purple).

The hardest thing about unconventional findings is not so much collecting the data but disrupting an established dogma

Malatesta and Gotz, Development 2013

Neurons derived from radial glial cells establish radial units in neocortex

Stephen C. Noctor*, Alexander C. Flint*, Tamily A. Weissman†, Ryan S. Dammerman†, & Arnold R. Kriegstein*†‡

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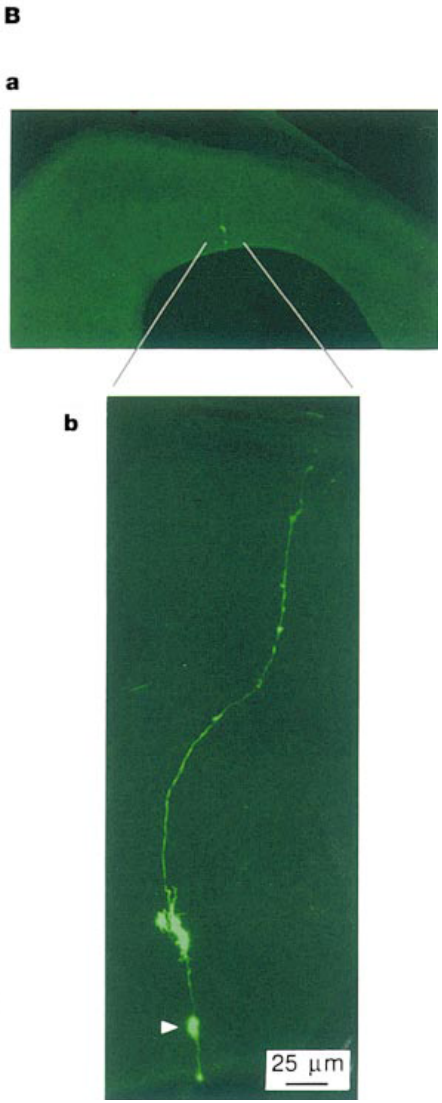
NATURE | VOL 409 | 8 FEBRUARY 2001 | www.nature.com



This study used retroviral labelling *in utero* and showed for the first time in vivo that radial glia are neuronal precursors

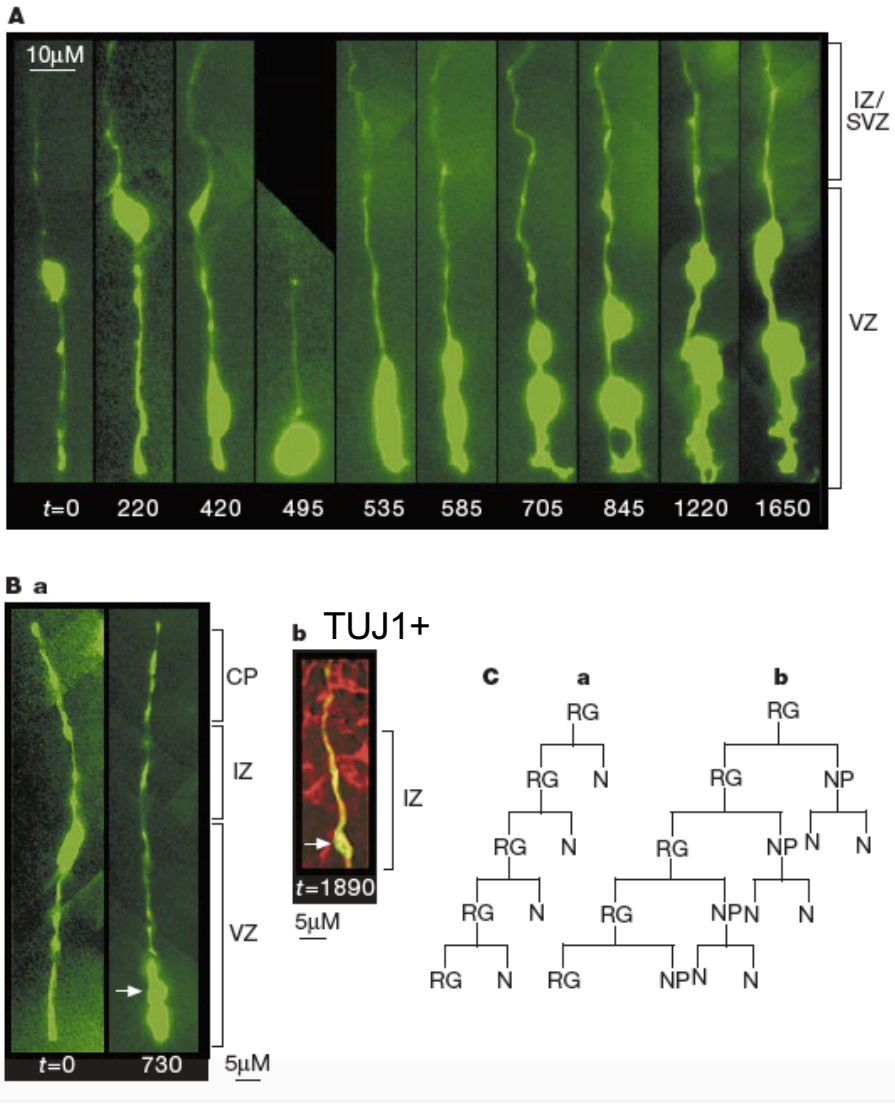
→ Intraventricular injections of green fuorescent protein (GFP)-expressing retrovirus to mark cortical precursor cells and their clonal progeny in E15-E16 rat embryos

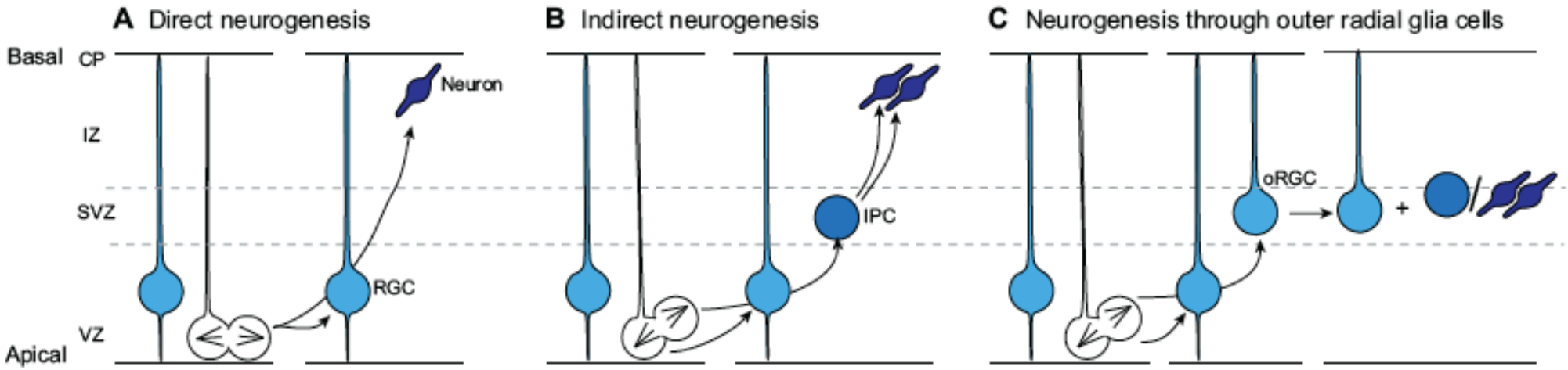
Time-lapse videomicroscopy of radial glial cell division

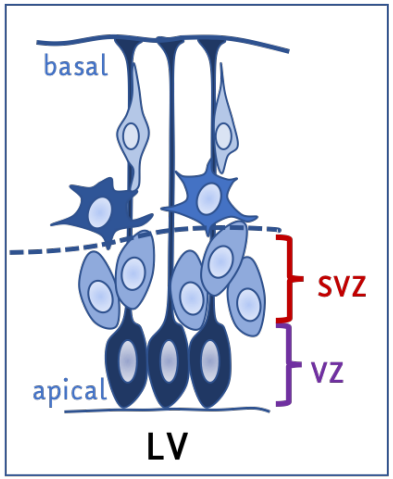
→ This experiment shows directly that radial glial cells can undergo asymmetrical division to generate neurons that migrate along the radial glial fibre.

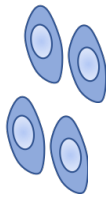
Watch the video @

http://ventricular.org/StephenNoctor/time-lapse-movies/









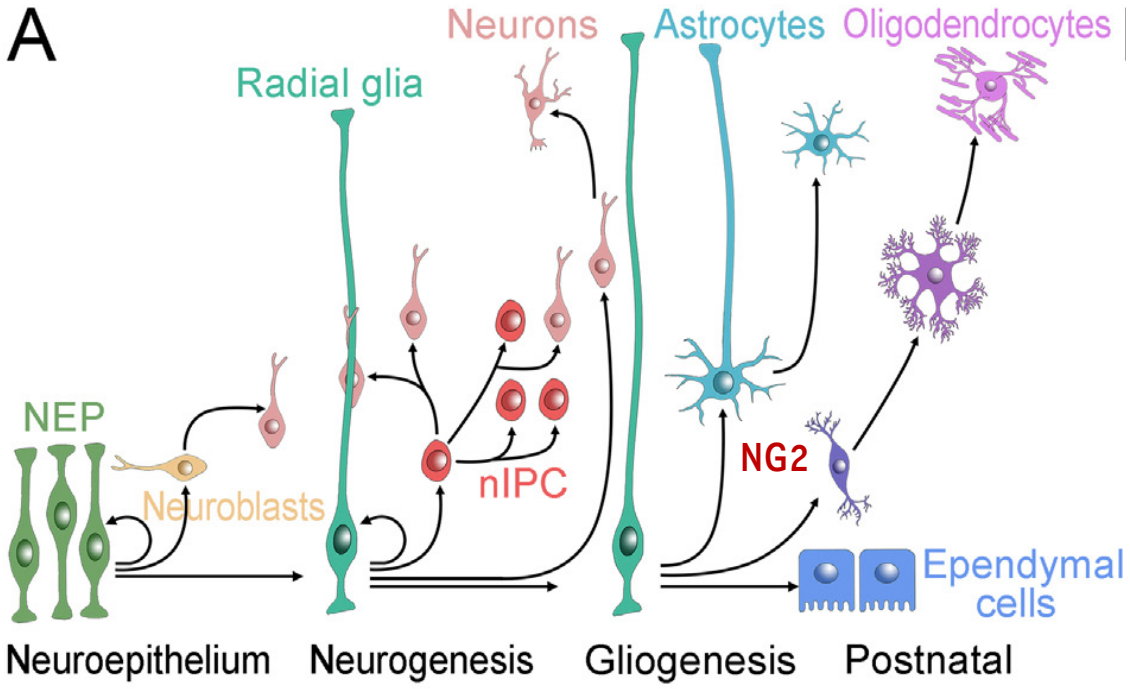
Tbr2 +

Intermediate progenitors cells (IPC)

or Basal progenitors

Fate of radial glial cells once neurogenesis is completed

- Depletion by symmetric differentiative division → generation of two postmitotic neurons
- Conversion to gliogenic function
- Differentiation into ependymal cells
- Transition to astrocytes (downregulation of Vimentin)
- Adult neurogenic astrocytes

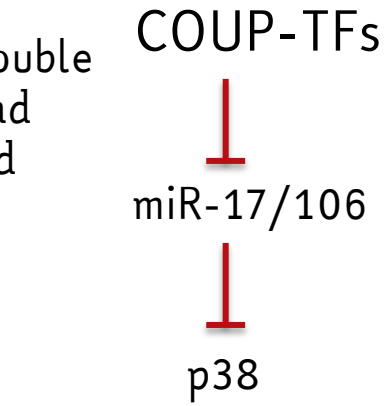


Astrocytes are first detected around E16 and oligodendrocytes around birth; however, the vast majority of both cell types are produced during the first month of postnatal development

Neural progenitor cells
competence change

Neurogenic → Gliogenic

- **TFs** (e.g. COUP-TFI and COUP-TFII critical molecular switches in the temporal identity transition of NSPCs, *Naka et al., 2008 Nat Neurosci*: the double knockdown of Coup-tfI/II in embryonic stem cell (ESC)-derived NSPCs and the developing mouse forebrain caused inhibition of gliogenesis, sustained neurogenesis and the prolonged generation of early-born neurons.
- **miRNA** → miR-17 and miR-106 downstream of COUP-TFs (miRNA overexpression → neurogenic phenotype) mediated by regulation of p38 (mitogen-activated protein kinase 14 or Mapk14 – p38 overexpression accelerate GFAP expression) (*Naka-Kaneda et al., 2014 PNAS*)



Neural progenitor cells
competence change

Neurogenic → Gliogenic

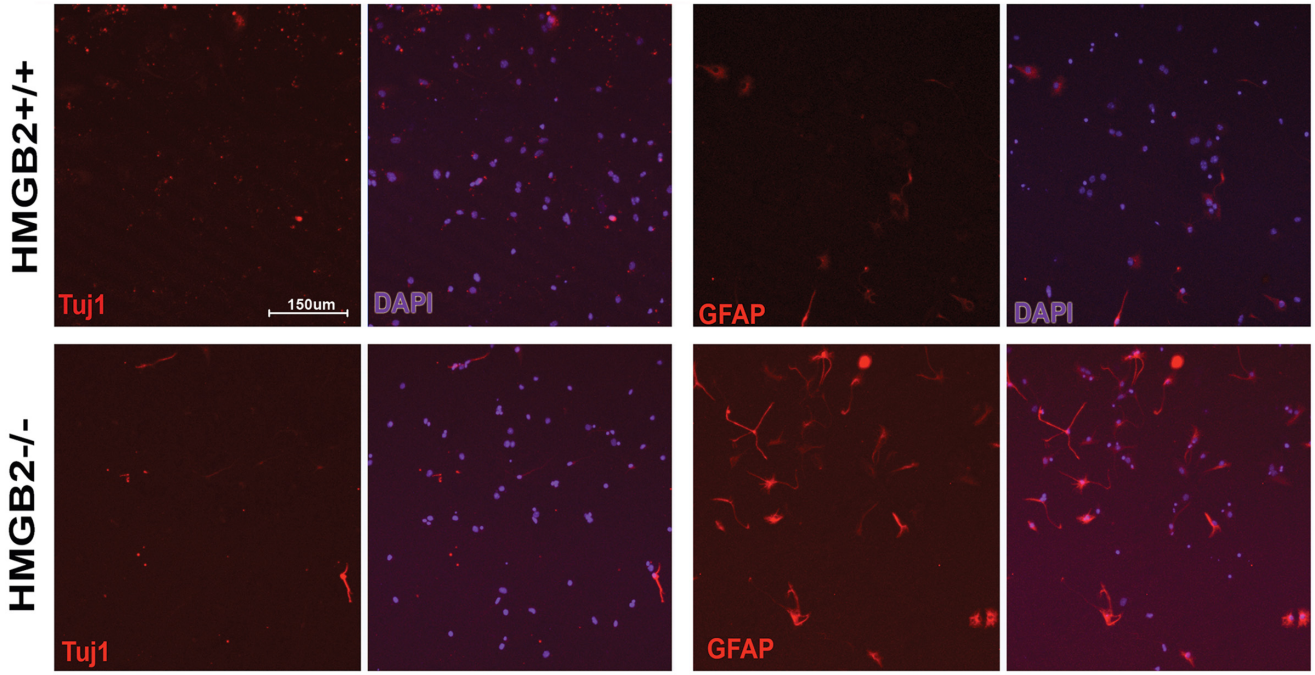
Epigenetic control

Chromatin binding proteins orchestrate the transcriptional repression of neuronal specific genes, while allowing for the activation of astrocyte-specific genes:

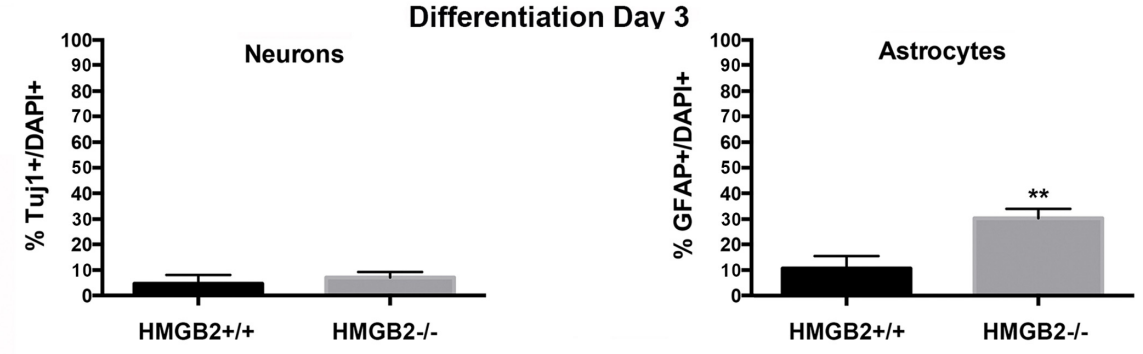
The chromatin protein high mobility group B2 (HMGB2), is dynamically expressed throughout embryonic development

In HMGB2 null NPCs the polycomb repressive complexes 1 and 2 (PRC1/2) are downregulated during the differentiation leading to downstream changes in the trimethylation of H3K27, essential for gene silencing and a subsequent shift (delay) in the neurogenesis to gliogenesis fate transition

(Bronstein et al., Front Mol Neurosci 2017)



Perinatal NSPCs monolayer cultures



Ratios of neural and glial cells altered in HMGB2 null SVZ cultures at day 3 of differentiation.
n = 3 biological replicates per genotype.

Sixty cells were counted per biological replicate. GFAP p = 0.0049.

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Perinatal NSPCs monolayer cultures

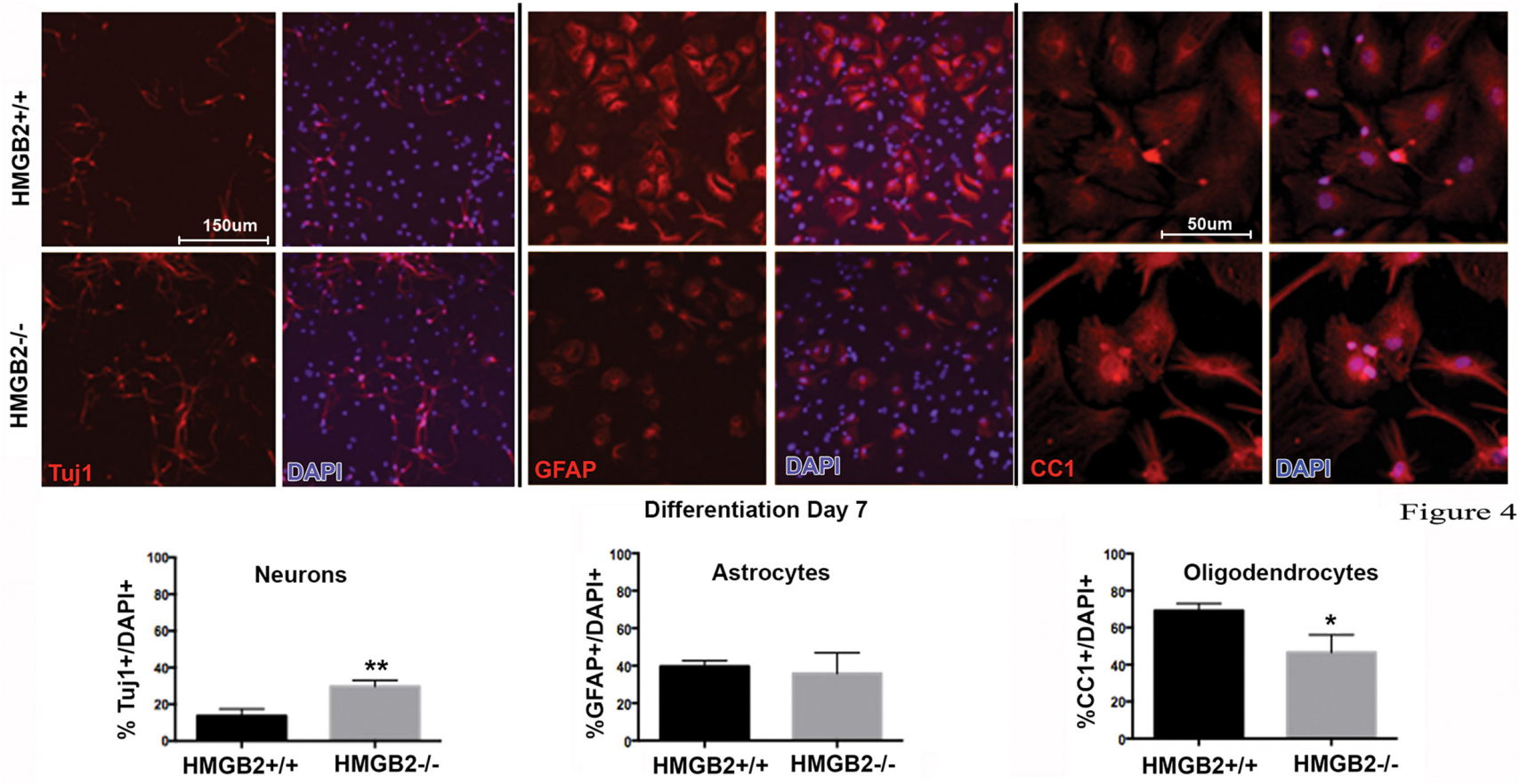


Figure 4

Ratios of neural and glial cell altered in HMGB2 null SVZ cultures at day 7 of differentiation.

n = 3 biological replicates per genotype, 60 cells were counted per biological replicate. Tuj1 p = 0.0054, CC1 p = 0.0189

Astrocytes (the most abundant macroglia)

ROS removal

Transmitters reuptake and release

Ion buffering

Secretion of trophic factors

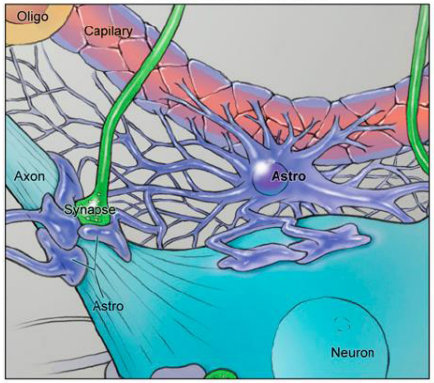
Antioxidant metabolism

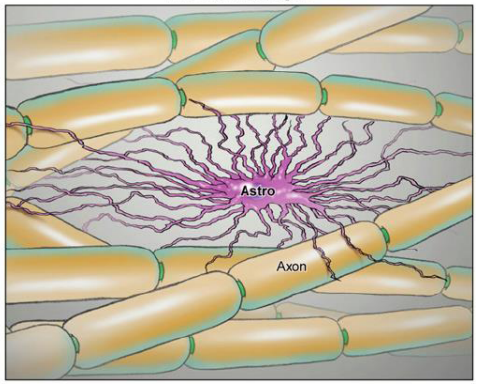
Blood brain barrier

classical view → two main sub-types

Protoplasmic **Fibrous**

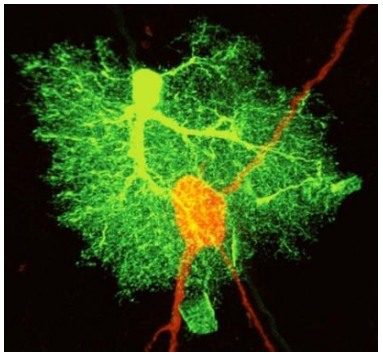
(gray matter - GM)

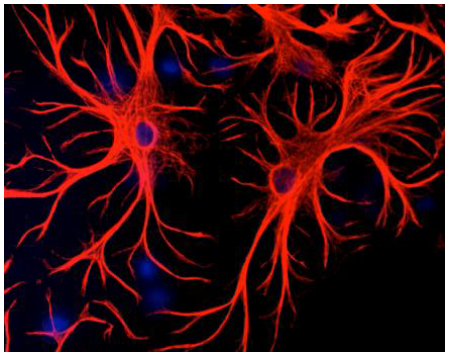




(white matter - WM)

Bushy morphology





Star-like morphology

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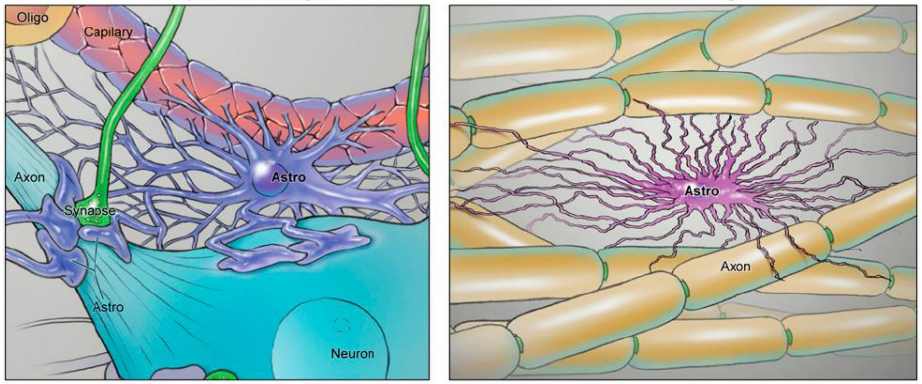
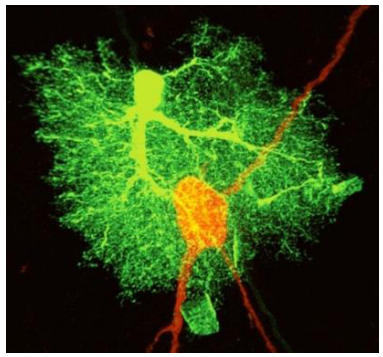
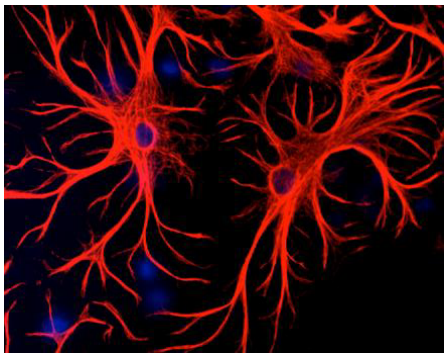
Astrocytes (the most abundant macroglia)

classical view → two main sub-types

Protoplasmic **Fibrous**







<u>Highly heterogeneous:</u>

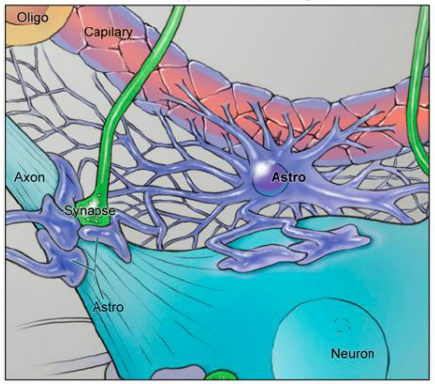
different sub-populations
in different brain regions

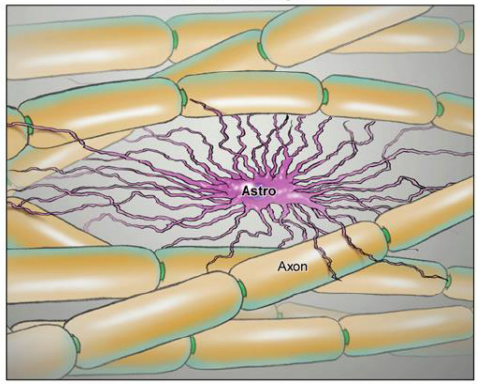
- morphological
- molecular
- biophysical
- functional

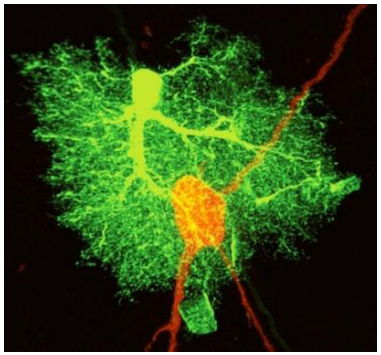
Astrocytes (the most abundant macroglia)

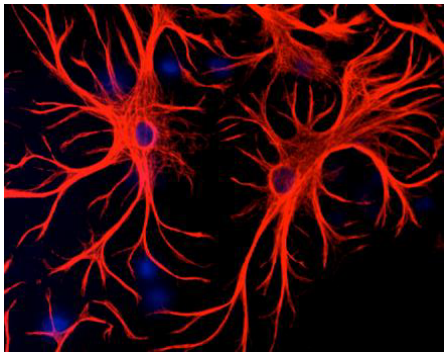
classical view → two main sub-types

Protoplasmic **Fibrous**









<u>Highly heterogeneous:</u>

different sub-populations

- morphological
- biochemical
- biophysical
- functional

Are the different sub-types generated from a homogeneous population of progenitors?
or from separated classes of progenitors?

Cerebral Cortex June 2013;23:1463–1472
doi:10.1093/cercor/bhs134
Advance Access publication May 22, 2012

Clonal Identity Determines Astrocyte Cortical Heterogeneity

Jorge García-Marqués and Laura López-Mascaraque

Department of Molecular, Cellular and Developmental Neurobiology, Instituto Cajal (CSIC), Madrid, Spain

Address correspondence to Laura López-Mascaraque, Instituto Cajal (CSIC), Av. Dr Arce, 37, 28002 Madrid, Spain.
Email: mascaraque@cajal.csic.es

Astrocytes are the most numerous cell type in the brain, where they are known to play multiple important functions. While there is increasing evidence of their morphological, molecular, and functional heterogeneity, it is not clear whether their positional and morphological identities are specified during brain development. We address this problem with a novel strategy to analyze cell lineages through the combinatorial expression of fluorescent proteins. Following in utero electroporation, stochastic expression of these proteins produces inheritable marks that enable the long-term in vivo tracing of glial progenitor lineages. Analyses of clonal dispersion in the adult cortex revealed unanticipated and highly specific clonal distribution patterns. In addition to the existence of clonal arrangements in specific domains, we found that different classes of astrocytes emerge from different clones. This reinforces the view that lineage origin impinges on cell heterogeneity, unveiling a new level of astrocyte diversity likely associated with specific regional functions.

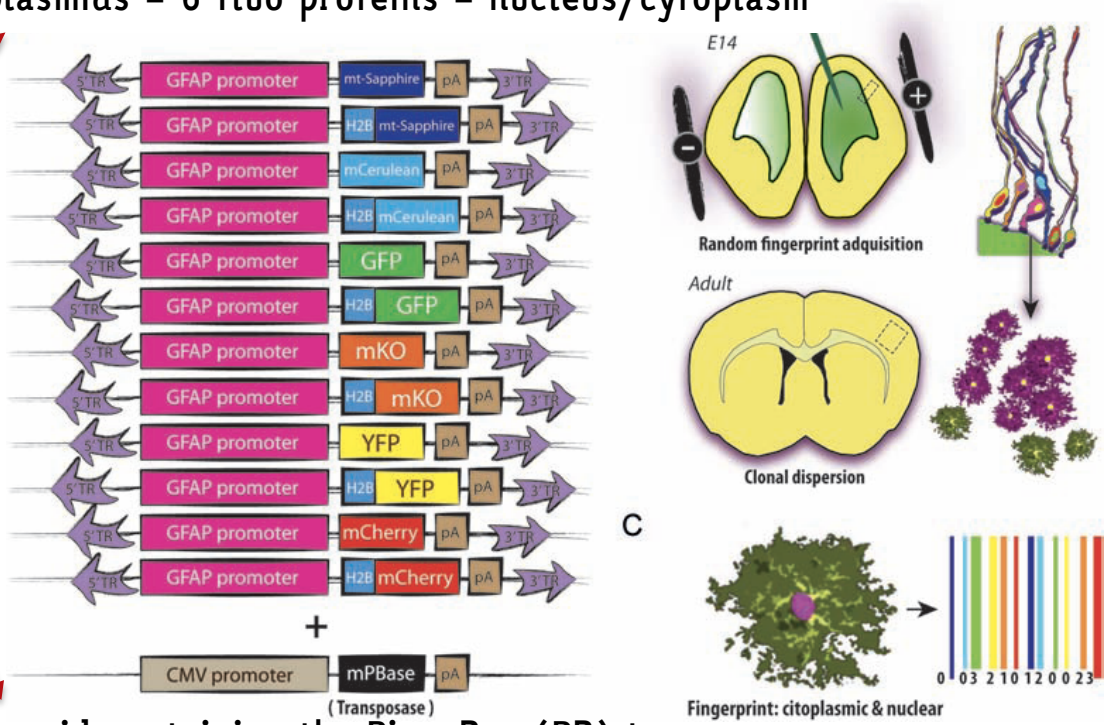
Keywords: glia, gliogenesis, lineage, pial, progenitor

Clonal cell lineage analysis defines
the potential of single cells
and the diversity they can produce

Star Track method – a genetic tracing strategy for long term lineage of individual glial progenitors in vivo -

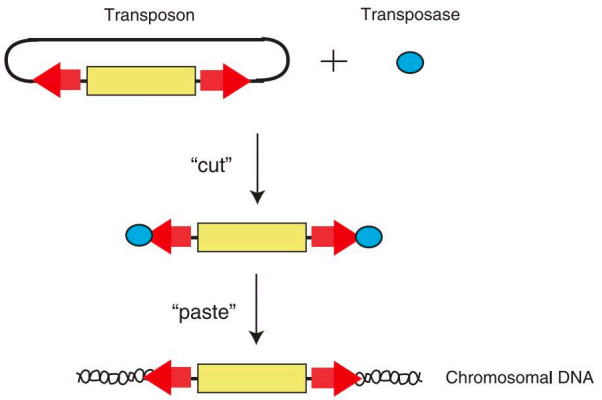
12 plasmids – 6 fluo proteins – nucleus/cytoplasm

Co-electroporation



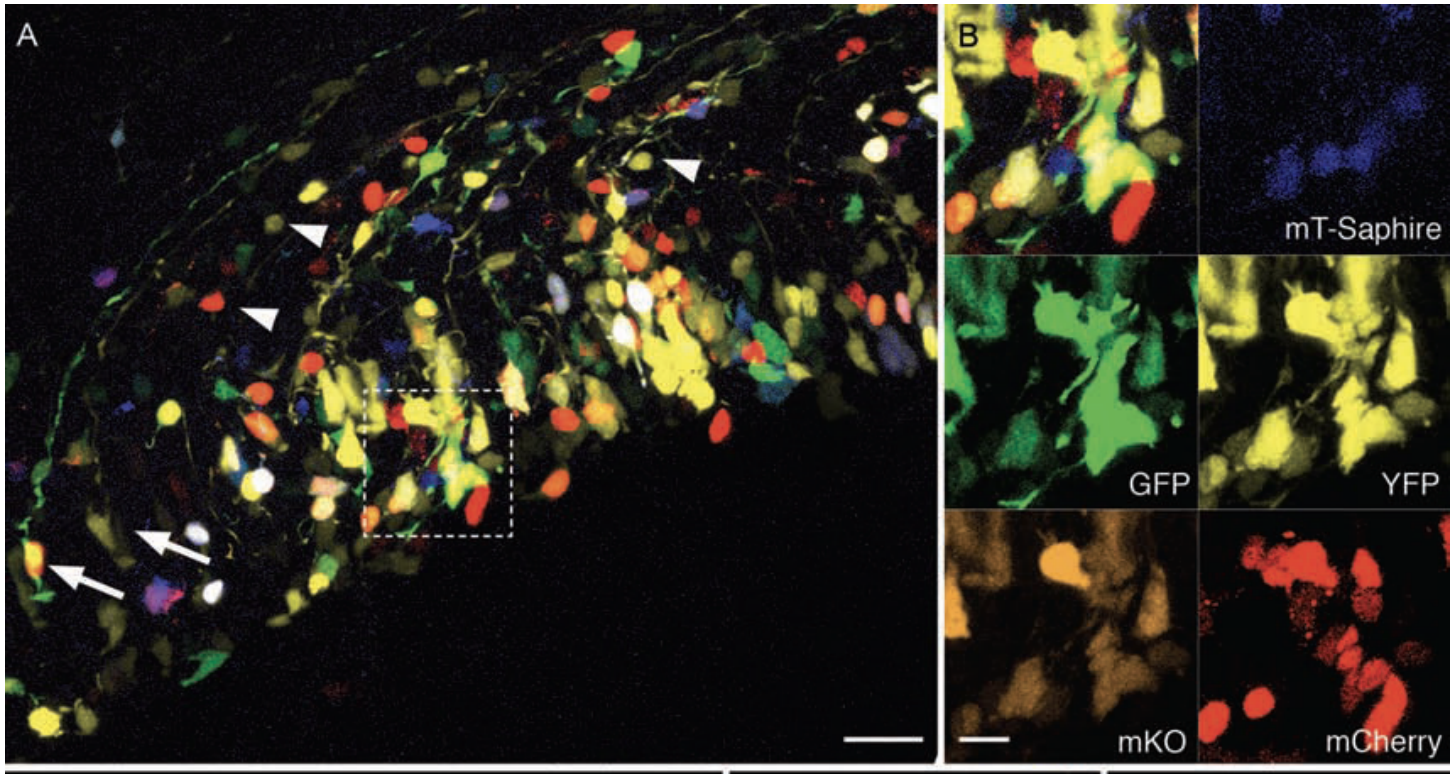
1 plasmid containing the PiggyBac (PB) transposase

Based on the **combinatorial expression** of different fluorescent proteins driven by the GFAP promoter following *in utero* electroporation (the construct corresponding to GFAP-XFP-pA was inserted between the inverted terminal repeats of the PiggyBac transposon)



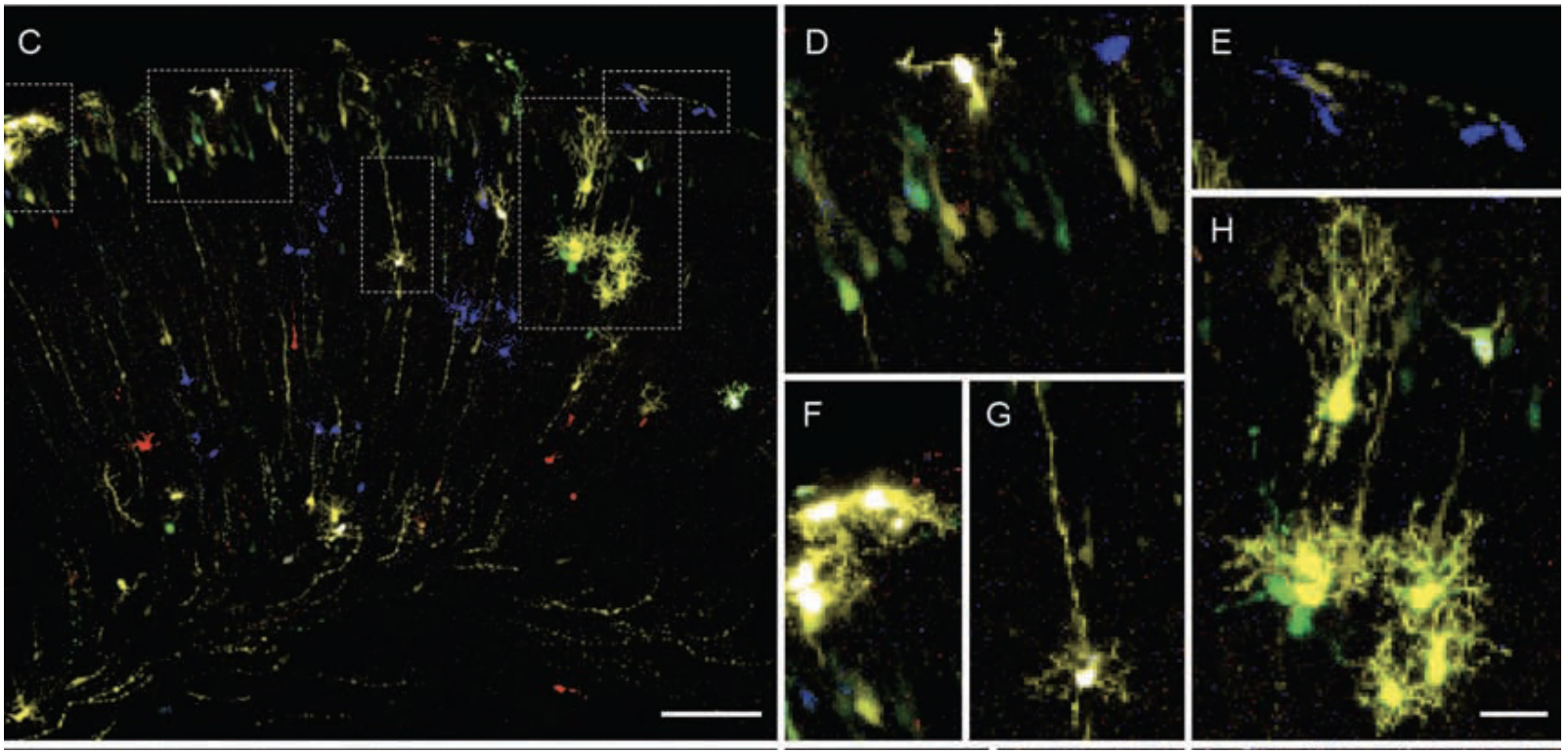
→ Following PBase-mediated transposition, fluorophores are randomly integrated into the cell genome

E18 cortex (electroporated at E14)



The large diversity of colors delineates the electroporation site

Each cell acquires a single color combination in the cytoplasm, nucleus, or both

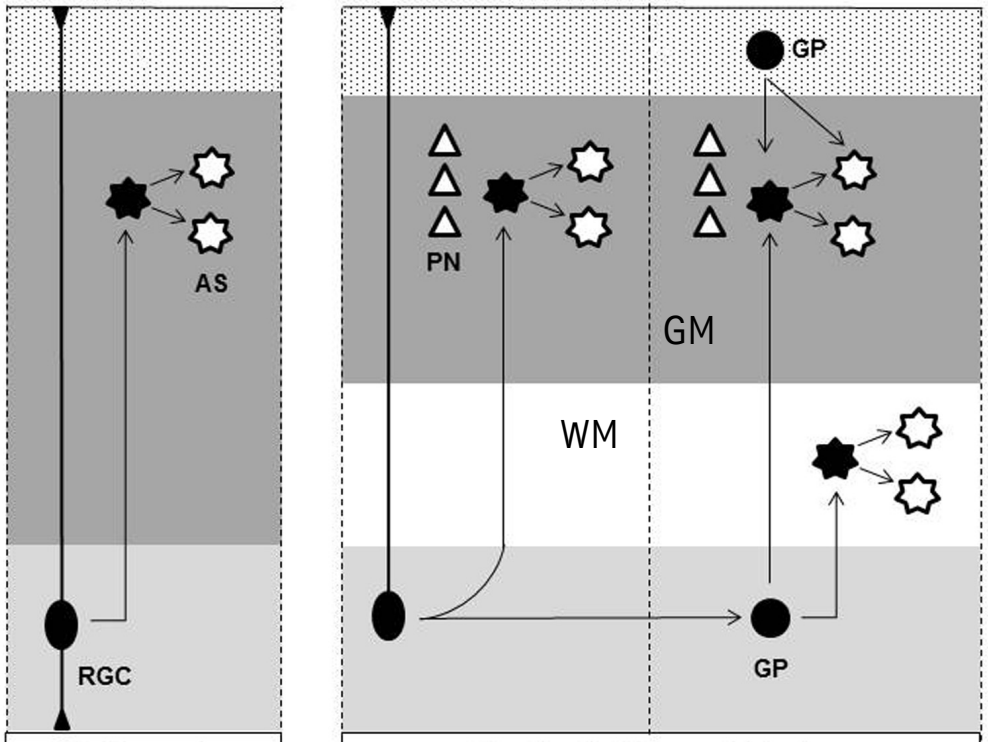


Clonally related astrocytes are spatially restricted

They disperse radially in the cortex spanning the entire depth of the cortical parenchyma

(D) Pyramidal-like cells mostly packed in layers 2/3 displaying faint fluorescence.
(E and F) Astrocyte clones confined to the pial brain surface.
(G) Radial glia transforming into astrocytes.
(H) Cell cluster with the same fluorescent mark formed by immature astrocytes/progenitors resembling protoplasmic astrocytes

Muliple origin of astrocytes in the cerebral cortex



Schitine et al., 2015

Transformation (direct)

RGCs ⟷ **Astrocytes**

Generation of intermediate progenitors (GPs) in the SVZ

Deep cortical layers 5/6 + WM astrocytes

GPs (marginal zone/layer I) ⟷ **Astrocytes**

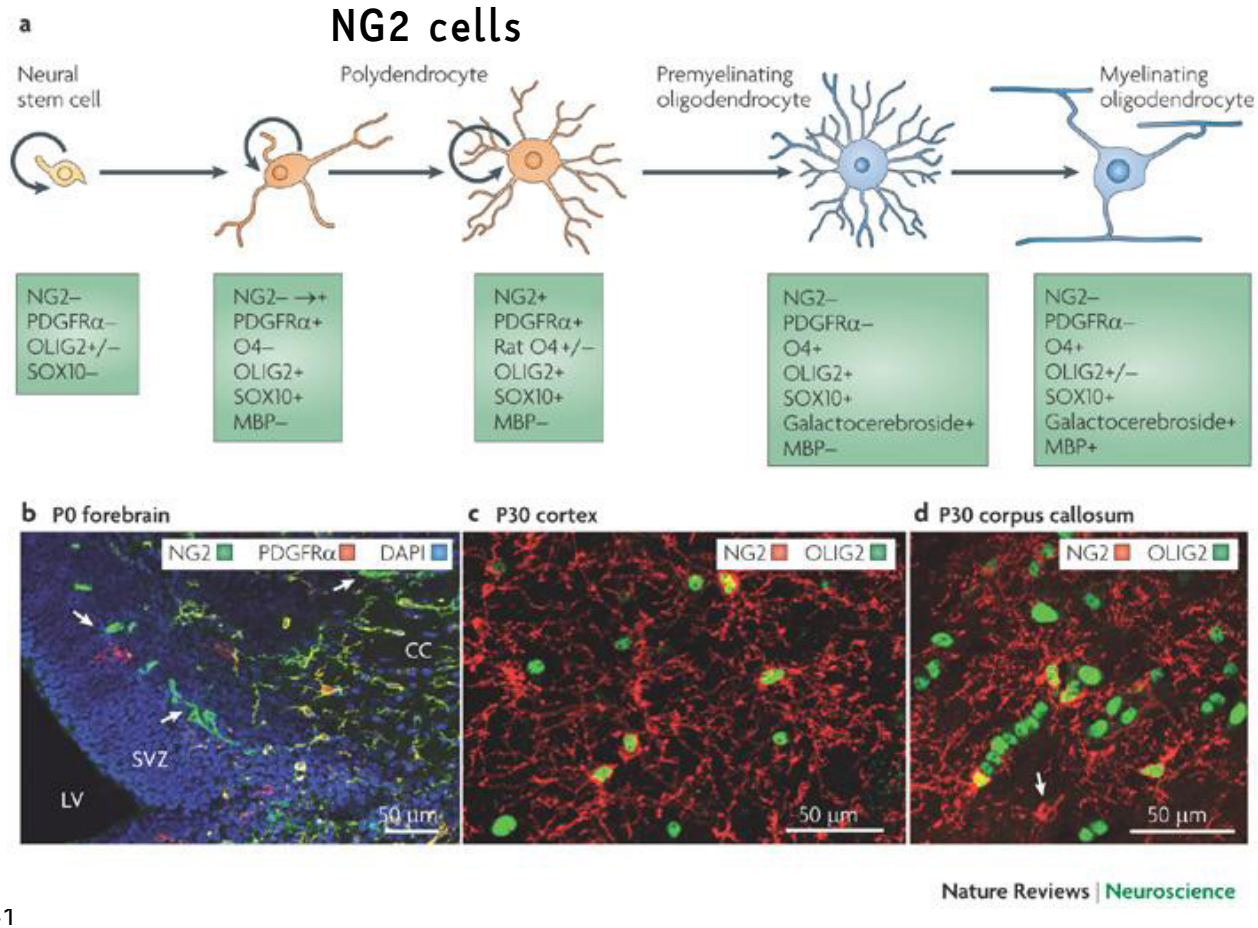
superficial astrocytes

Immature astrocytes (superficial layers) ⟷ **Astrocytes**

Superficial cortical layers 1/4 astrocytes

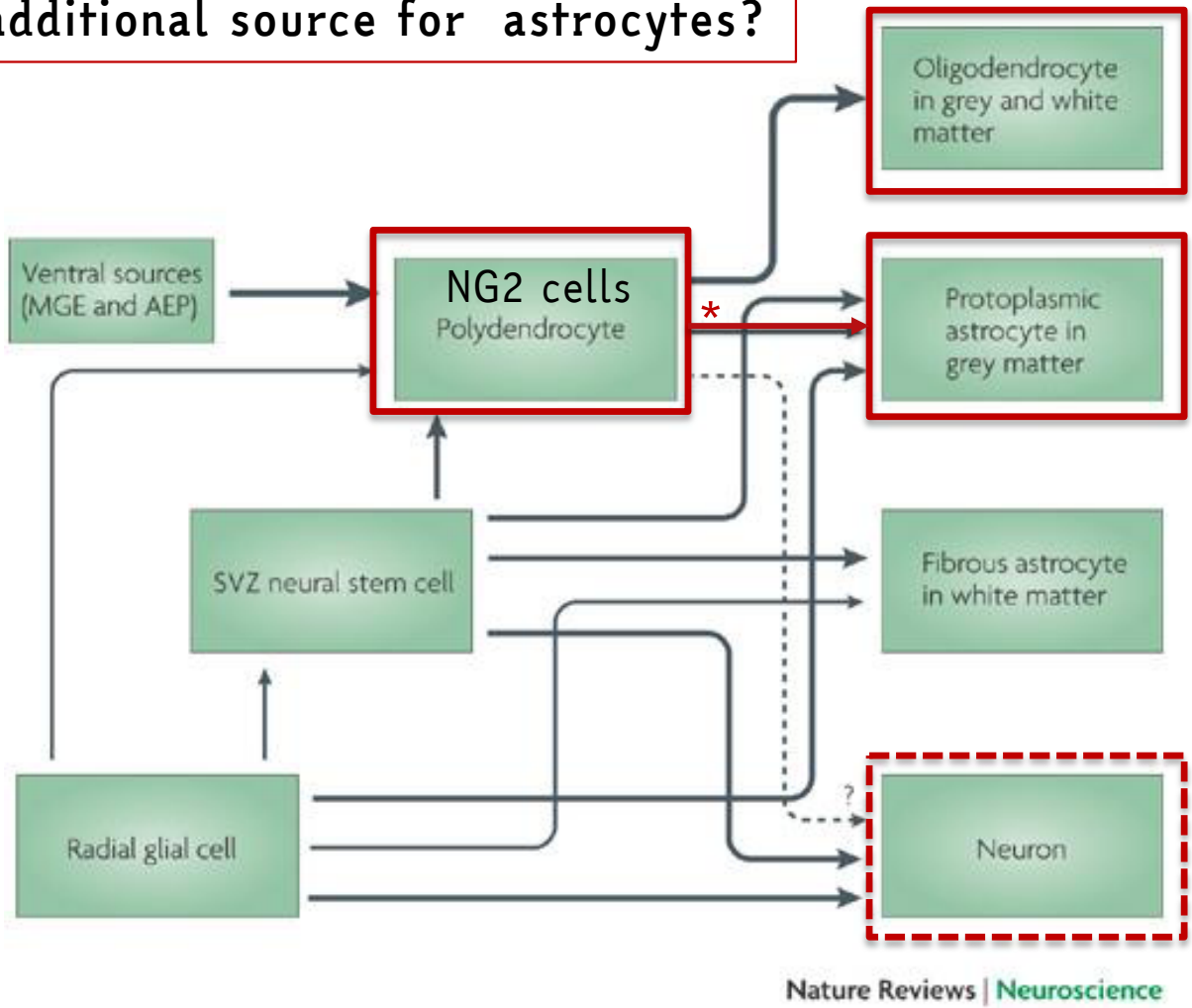
start@unito

NG2 cells generate **oligodendrocytes** in the developing and mature CNS



whether NG2+ cells are multipotential cells that can give rise to neurons and astrocytes is now highly debated

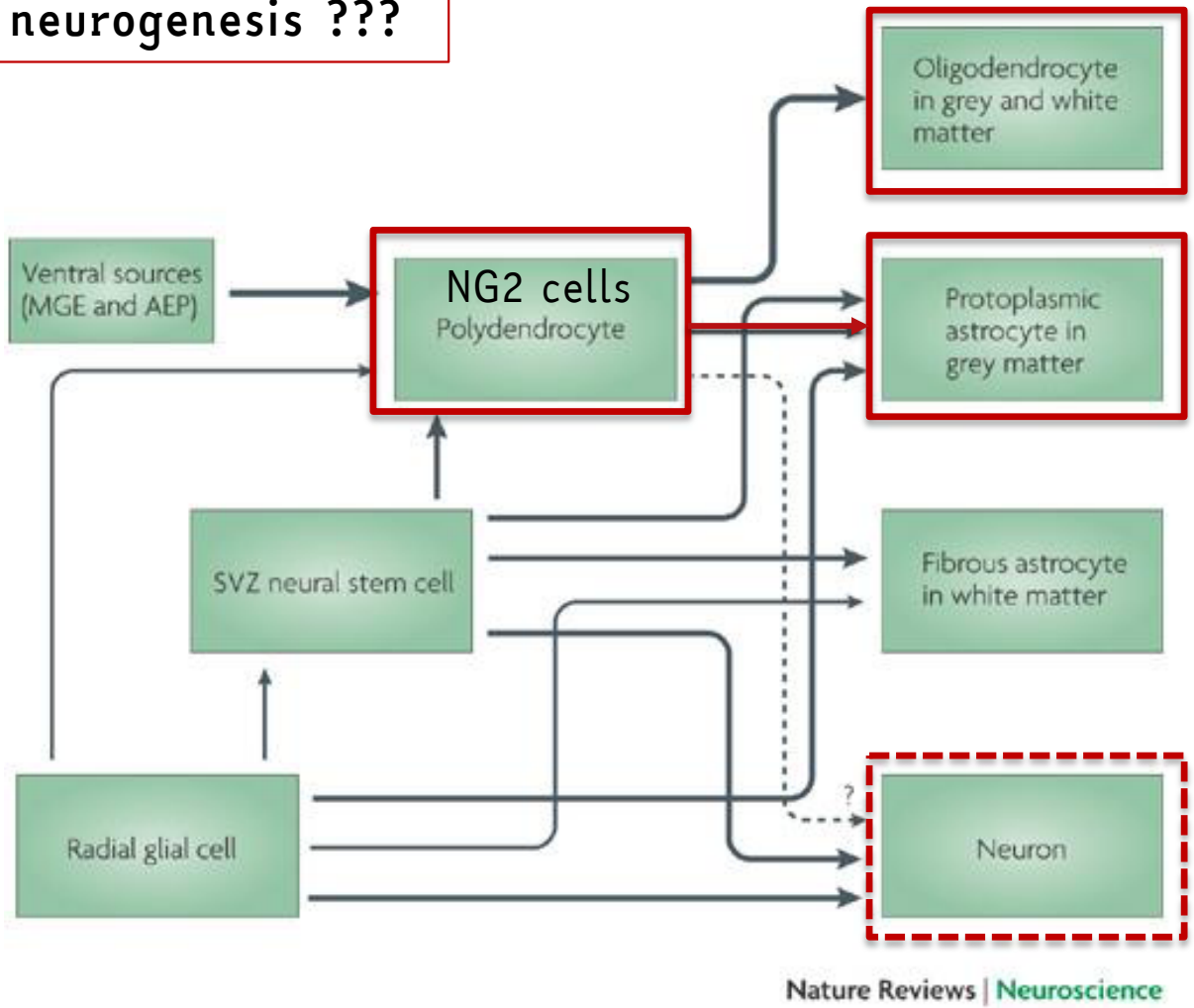
NG2 cells an additional source for astrocytes?



* only in ventral forebrain

NG2 astrogliogenic potential is temporally and spatially restricted to a subset of ventral embryonic progenitors

NG2 cells and neurogenesis ???



Glia. 2019 Jun;67(6):1094-1103. doi: 10.1002/glia.23590. Epub 2019 Feb 6.

Early embryonic NG2 glia are exclusively gliogenic and do not generate neurons in the brain.

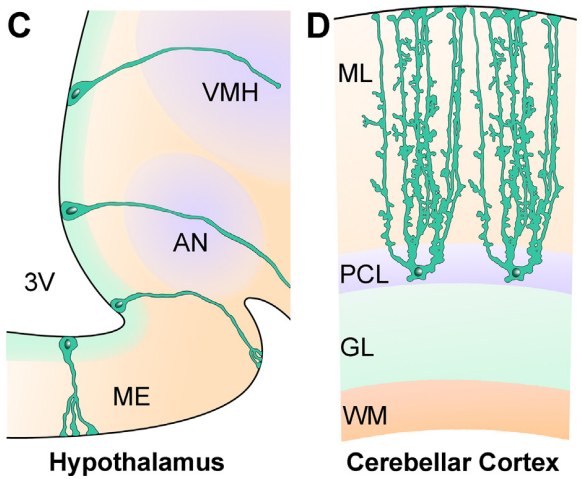
Huang W[1], Guo Q[1], Bai X[1], Scheller A[1], Kirchhoff F[1].

Author information

Abstract

In the central nervous system, the type I transmembrane glycoprotein NG2 (nerve-glia antigen 2) is only expressed by pericytes and oligodendrocyte precursor cells (OPCs). Therefore, OPCs are also termed NG2 glia. Their fate during development has been investigated systematically in several genetically modified mouse models. Consensus exists that postnatal NG2 glia are restricted to the oligodendrocyte (OL) lineage, while, at least in the forebrain, embryonic NG2 glia could also generate astrocytes. In addition, experimental evidence for a neurogenic potential of NG2 glia in the early embryonic brain (before E16.5) has been provided. However, this observation is still controversial. Here, we took advantage of reliable transgene expression in NG2-EYFP and NG2-CreERT2 knock-in mice to study the fate of early embryonic NG2 glia. While pericytes were the main cells with robust NG2 gene activity at E12.5, only a few OPCs expressed NG2 at this early stage of embryogenesis. Subsequently, this proportion of OPCs increased from 3% (E12.5) to 11% and 25% at E14.5 and E17.5, respectively. When Cre DNA recombinase activity was induced at E12.5 and E14.5 and pups were analyzed at postnatal day 0 (P0) and P10, the vast majority of recombined cells, besides pericytes, belonged to the OL lineage cells, with few astrocytes in the ventral forebrain. In other brain regions such as brain stem, cerebellum, and olfactory bulb only OL lineage cells were detected. Therefore, we conclude that NG2 glia from early embryonic brain are restricted to a gliogenic fate and do not differentiate into neurons after birth.

Radial glial-like subtypes in the adult CNS derived from embryonic RGCs

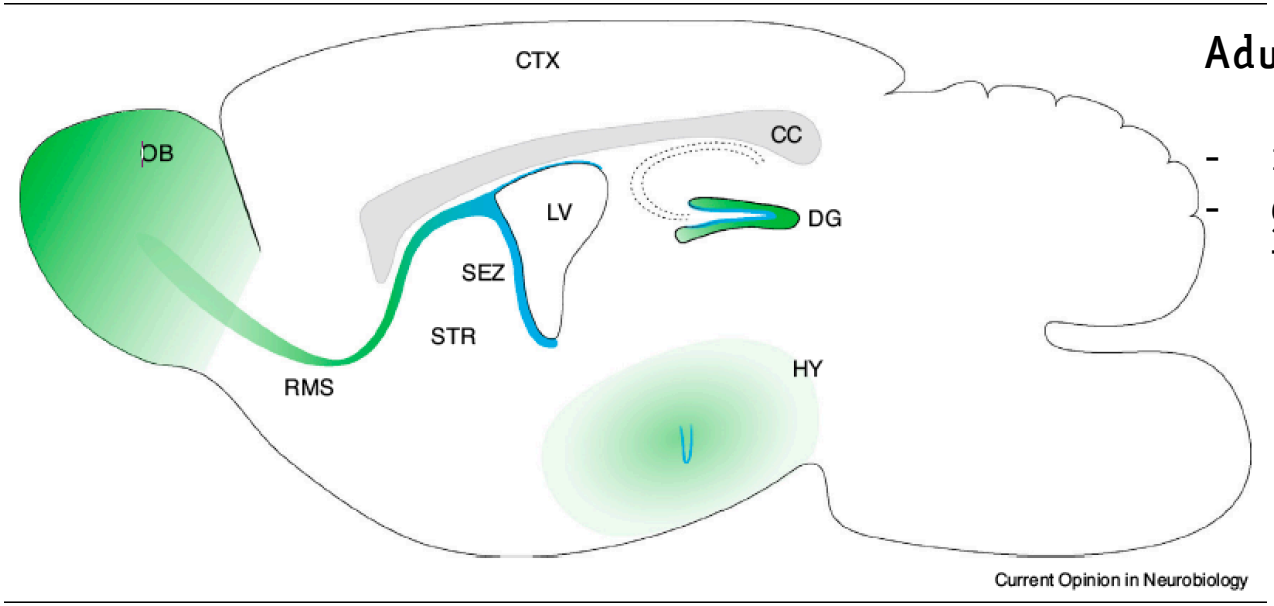


Tanycytes

Bergman glia

+ adult neurogenic niches....

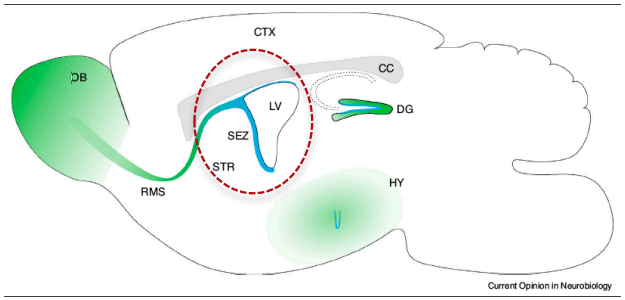
Location of adult neural proliferative zones



Falk and Gotz, 2017

Adult NSCs retain RGC hallmarks

- radial morphology
- gene expression profiles similar to their embryonic counterparts



Arturo Alvarez-Buylla
UCSF – San Francisco

neurons

glia ⟵ **RGCs** ⟶ aNSCs

Merkle et al., PNAS 2004

limited self-renewal capacity

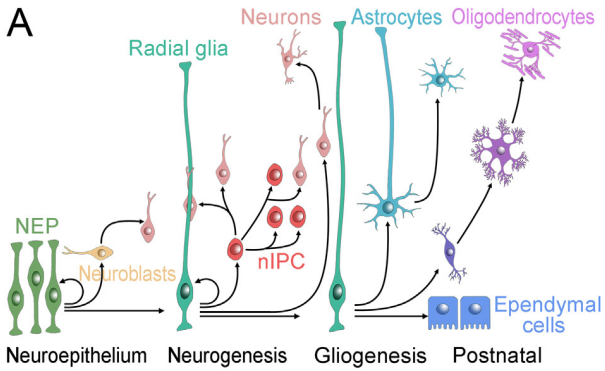
fast exhaustion

Embryonic development ----------> adult

How?

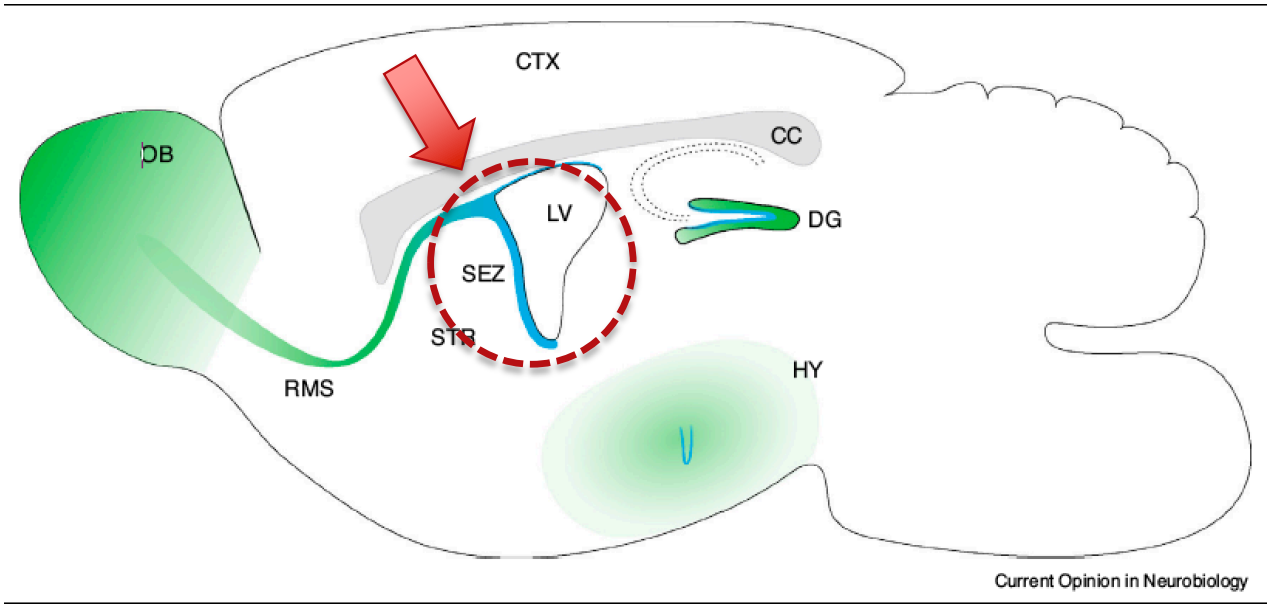
When?

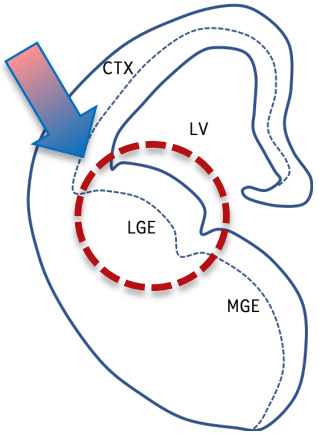
Embryonic development ----------> adult



Location of adult neural proliferative zones

The adult NSCs residing in the SEZ are mainly derived from progenitor cells in the LGE



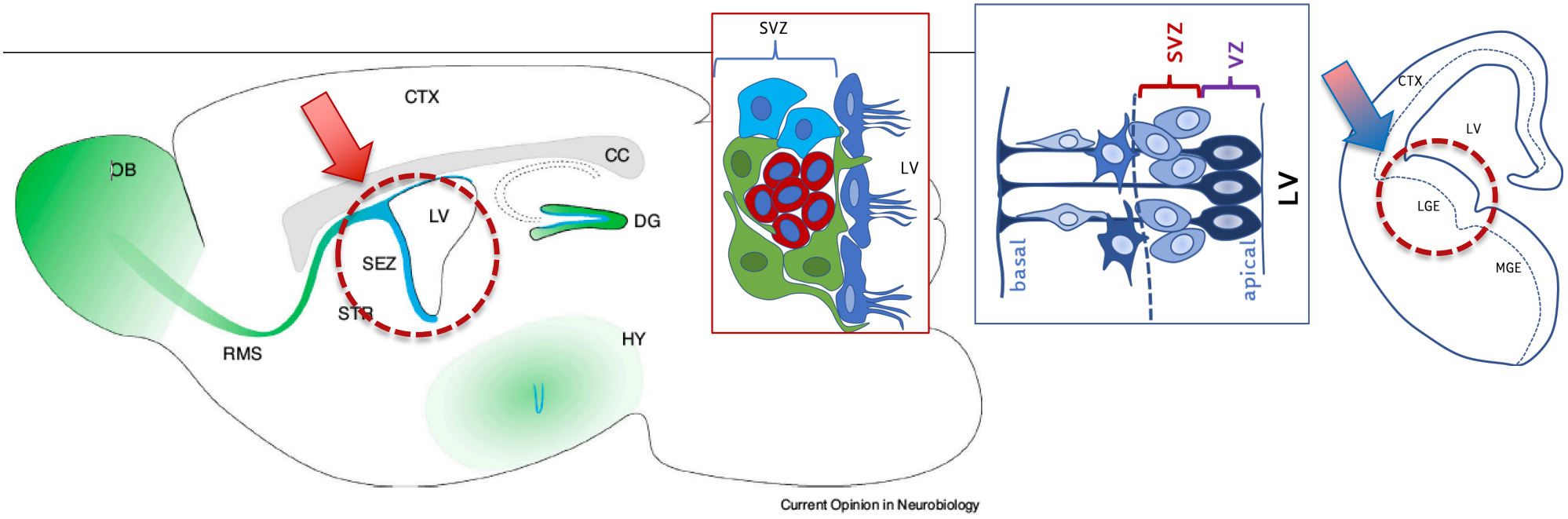


SEZ = subependymal zone

Falk and Gotz, 2017

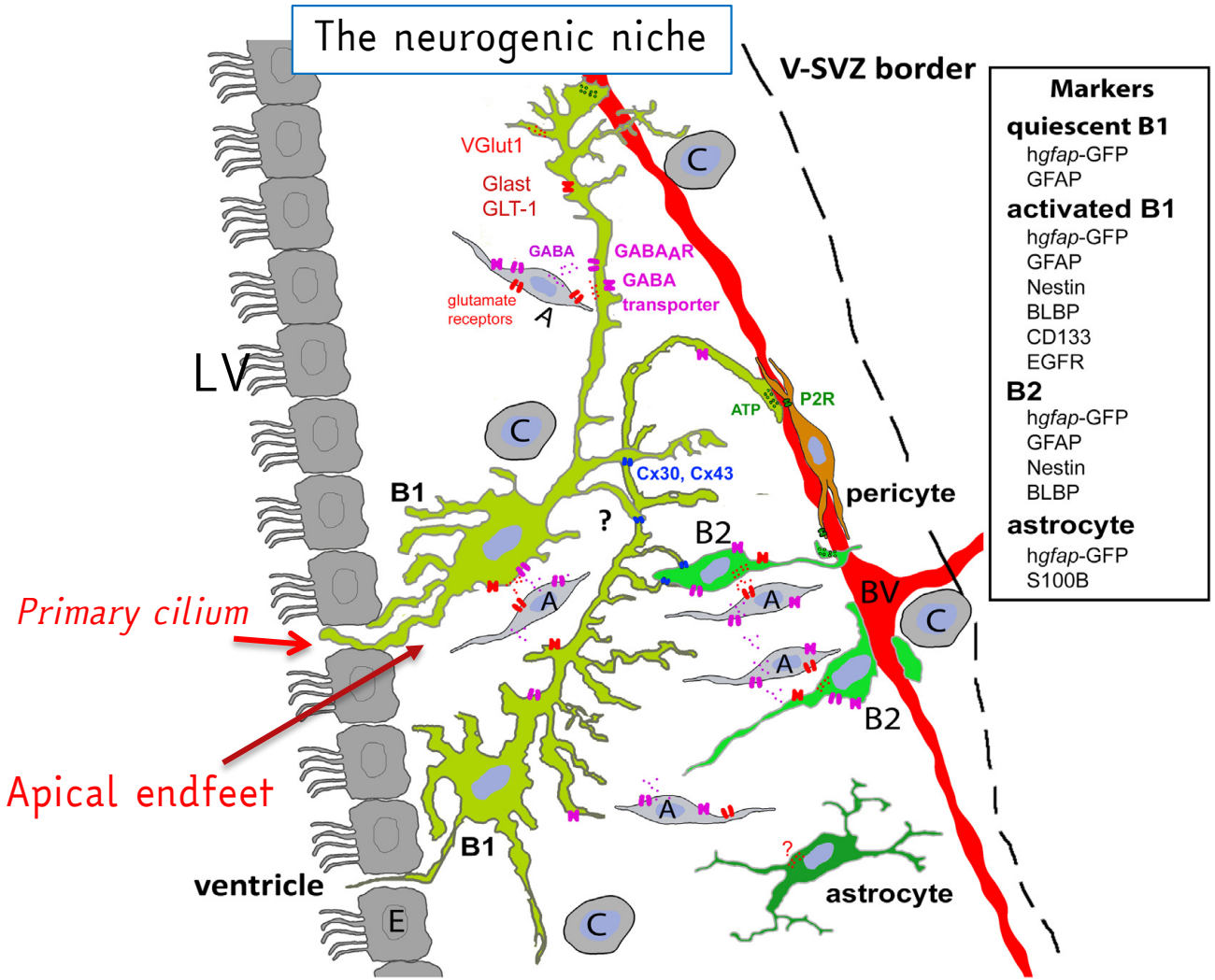
Location of adult neural proliferative zones

The adult NSCs residing in the SEZ are mainly derived from progenitor cells in the LGE



SEZ = subependymal zone

Falk and Gotz, 2017



B1 radial glia-like morphology

Cell 2015

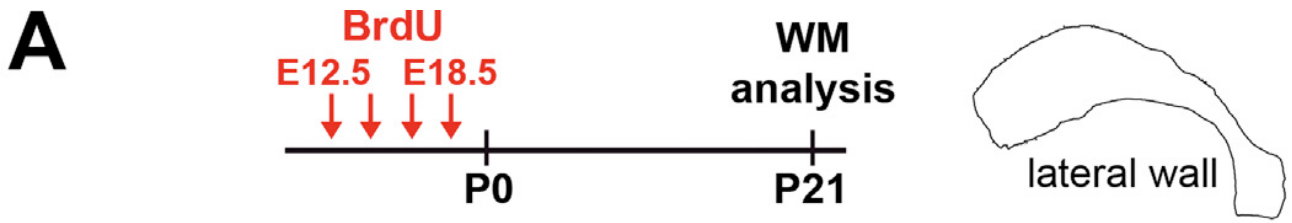
Article

When?

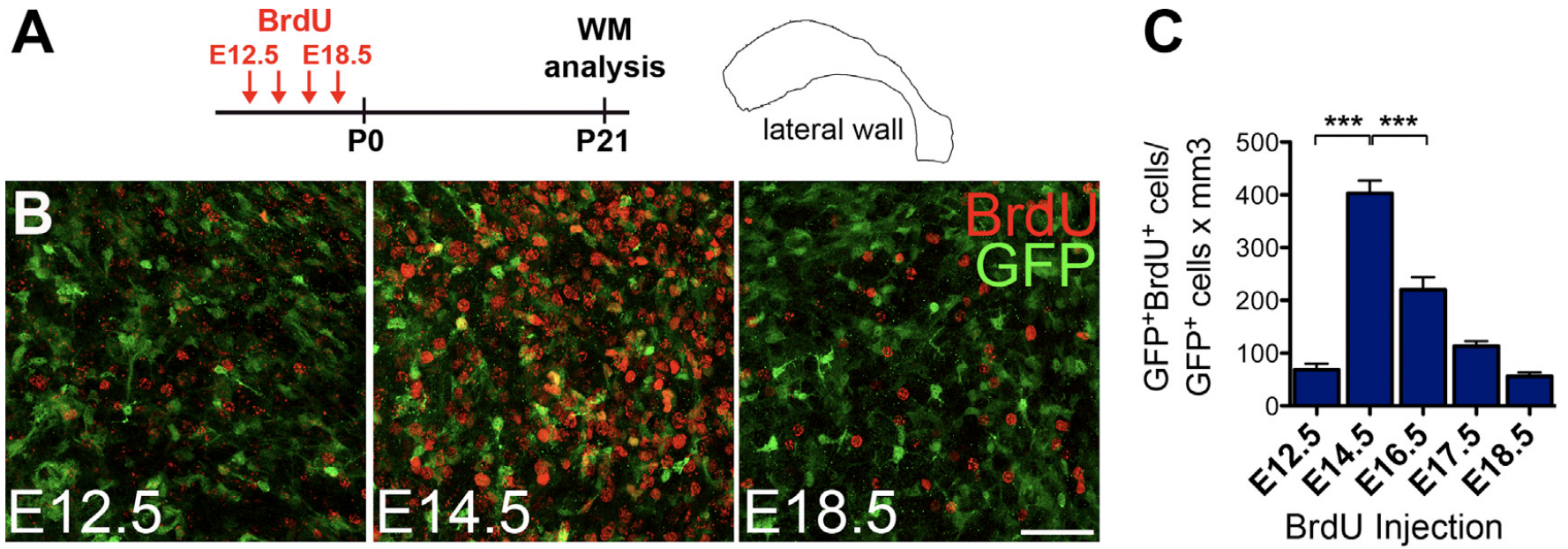
Embryonic Origin of Postnatal Neural Stem Cells

Luis C. Fuentealba,[1] Santiago B. Rompani,[2] Jose I. Parraguez,[1] Kirsten Obernier,[1] Ricardo Romero,[1] Constance L. Cepko,[2] and Arturo Alvarez-Buylla[1,*]
[1]Department of Neurological Surgery and the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA 94143, USA
[2]Departments of Genetics and Ophthalmology and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: abuylla@stemcell.ucsf.edu
http://dx.doi.org/10.1016/j.cell.2015.05.041

To address the contribution of dividing RGs at different stages of development to the postnatal day 21 (P21) B1 cell population, they injected timed pregnant **hGFAP::GFP mice** at different embryonic stages with the thymidine analog bromodeoxyuridine (BrdU)



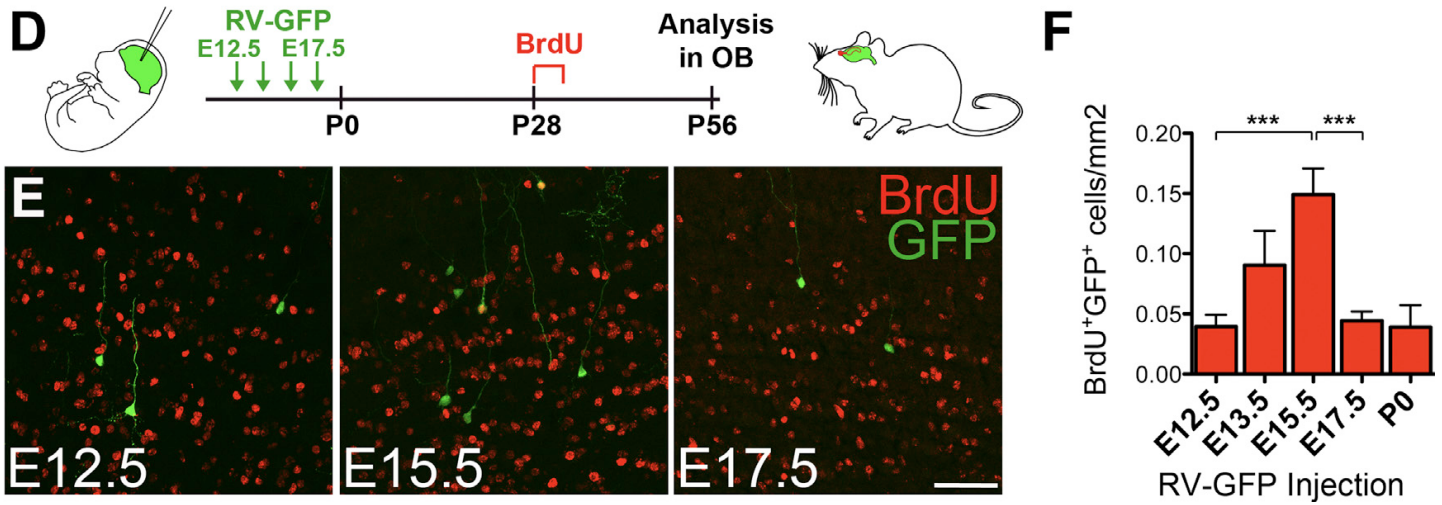
start@unito



The majority of double-labeled BrdU+/GFP+ B1 cells in the P21 V-SVZ were derived from animals injected with BrdU at E14.5 and E16.5

→ the majority of B1 cells were derived from RGs that divided during this period

Many of the B1 cells were **brightly labeled**, suggesting that they did not divide repeatedly in the intervening period between the time of BrdU injection in the embryo and P21

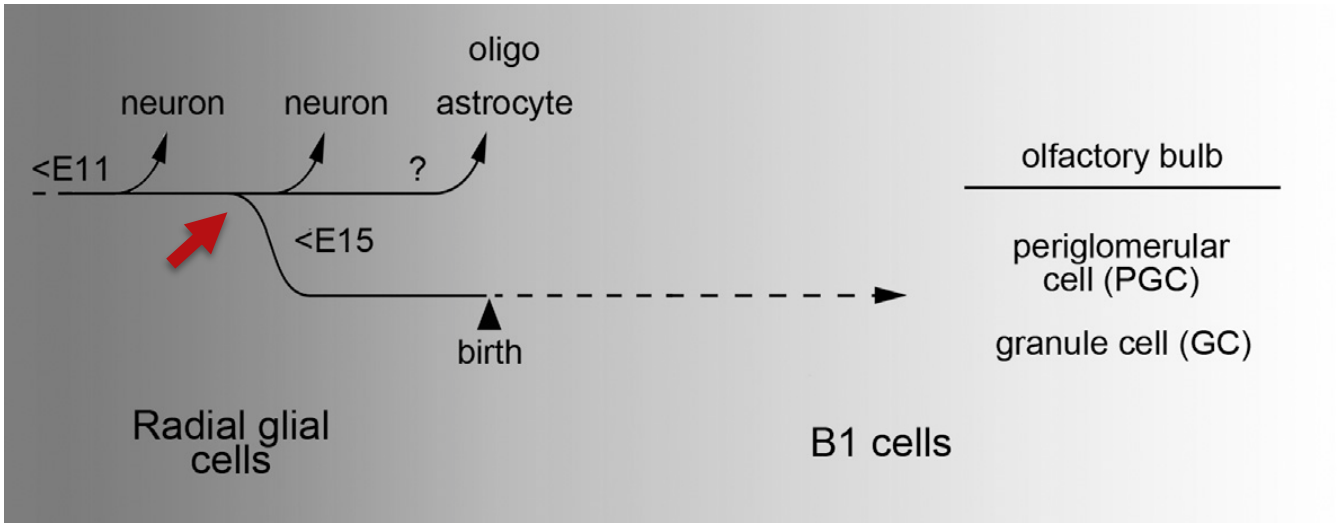


Alternative approach: injection of GFP-expressing retroviruses to transduce dividing embryonic VZ progenitors at different ages to permanently label their progeny in the postnatal brain.

The majority of GFP+ / BrdU+ OB interneurons were derived from progenitor cells that divided between E13.5–E15.5.

When?

These experiments suggest that **postnatal B1 cells are derived from** embryonic cells that divide during **mid-fetal development** and then remain relatively quiescent until they become reactivated to generate progeny in the postnatal brain



How?

Neuron. 2017 Feb 22;93(4):777-791.e3. doi: 10.1016/j.neuron.2017.02.009.

Time-Specific Effects of Spindle Positioning on Embryonic Progenitor Pool Composition and Adult Neural Stem Cell Seeding.

Falk S[1], Bugeon S[2], Ninkovic J[1], Pilz GA[3], Postiglione MP[4], Cremer H[2], Knoblich JA[4], Götz M[5].

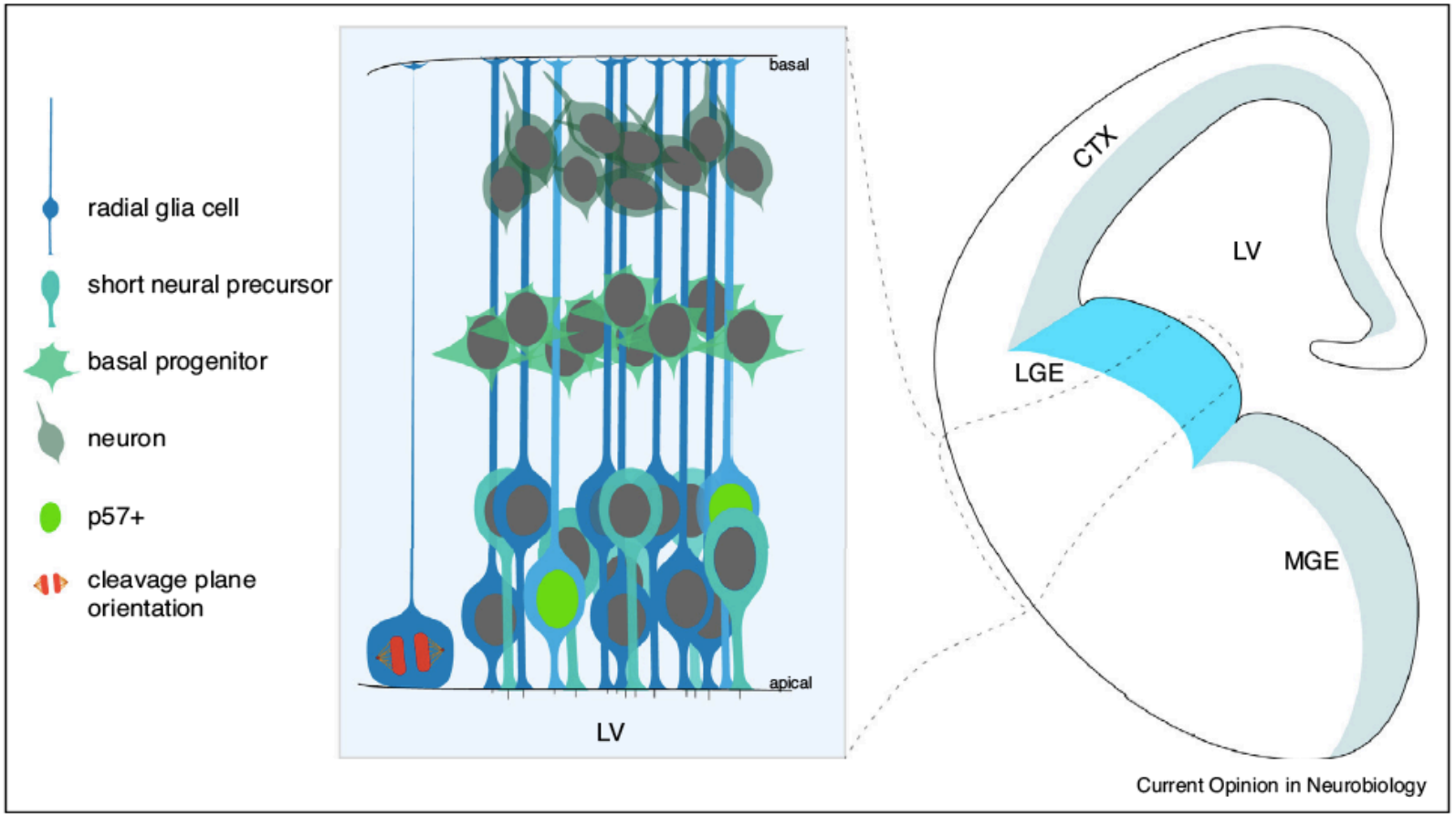
Author information

Abstract

The developmental mechanisms regulating the number of adult neural stem cells (aNSCs) are largely unknown. Here we show that the cleavage plane orientation in murine embryonic radial glia cells (RGCs) regulates the number of aNSCs in the lateral ganglionic eminence (LGE). Randomizing spindle orientation in RGCs by overexpression of Insc or a dominant-negative form of Lgn (dnLgn) reduces the frequency of self-renewing asymmetric divisions while favoring symmetric divisions generating two SNPs. Importantly, these changes during embryonic development result in reduced seeding of aNSCs. Interestingly, no effects on aNSC numbers were observed when Insc was overexpressed in postnatal RGCs or aNSCs. These data suggest a new mechanism for controlling aNSC numbers and show that the role of spindle orientation during brain development is highly time and region dependent.

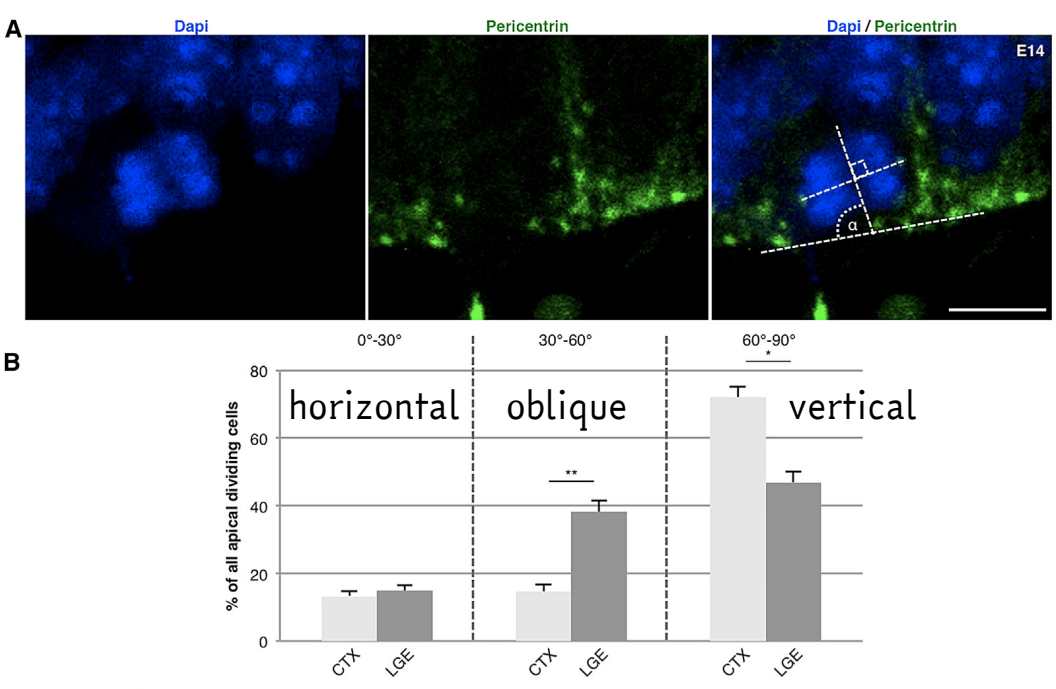
The control of the division type in progenitor cells during embryonic development (in a specific time window) regulates the number of embryonic progenitor cells destined to become adult neural stem cells

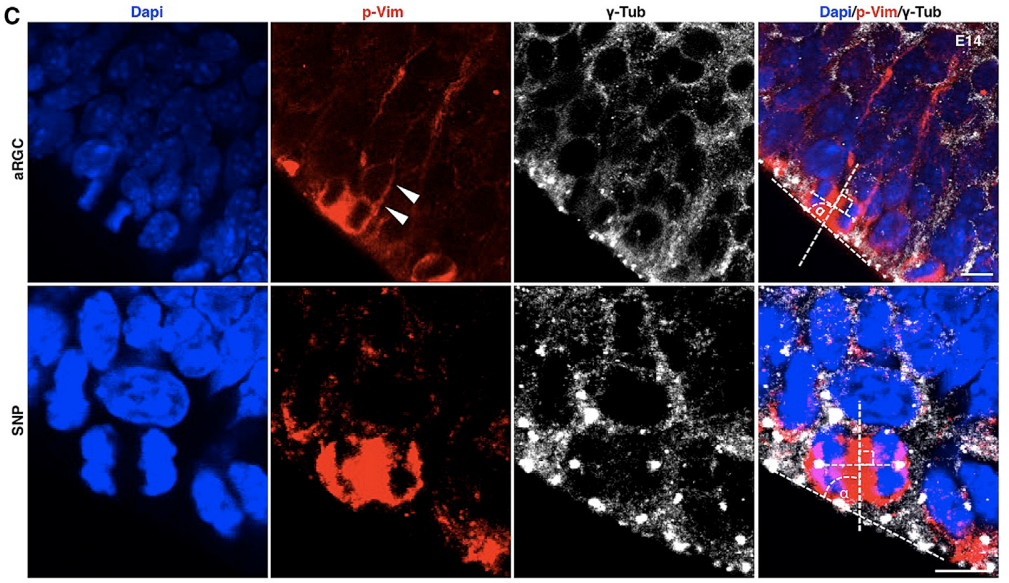
How?

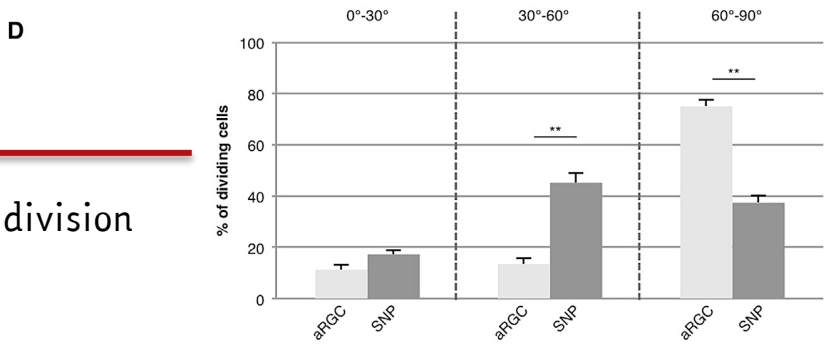


Embryonic origin of adult neural stem cells

Spindle positioning during M-phase is implicated in the regulation of the size of stem and progenitor cell pools in a multitude of tissues by coordinating the mode of cell division







In the lGE: two types of dividing progenitors → different division plane

RGCs = cells with a basal process (arrowhead)
SNP= short neural progenitor cells - cells without a basal process during mitosis (specific class of transient amplifying progenitors)

progenitor subtype-specific bias for cleavage angles

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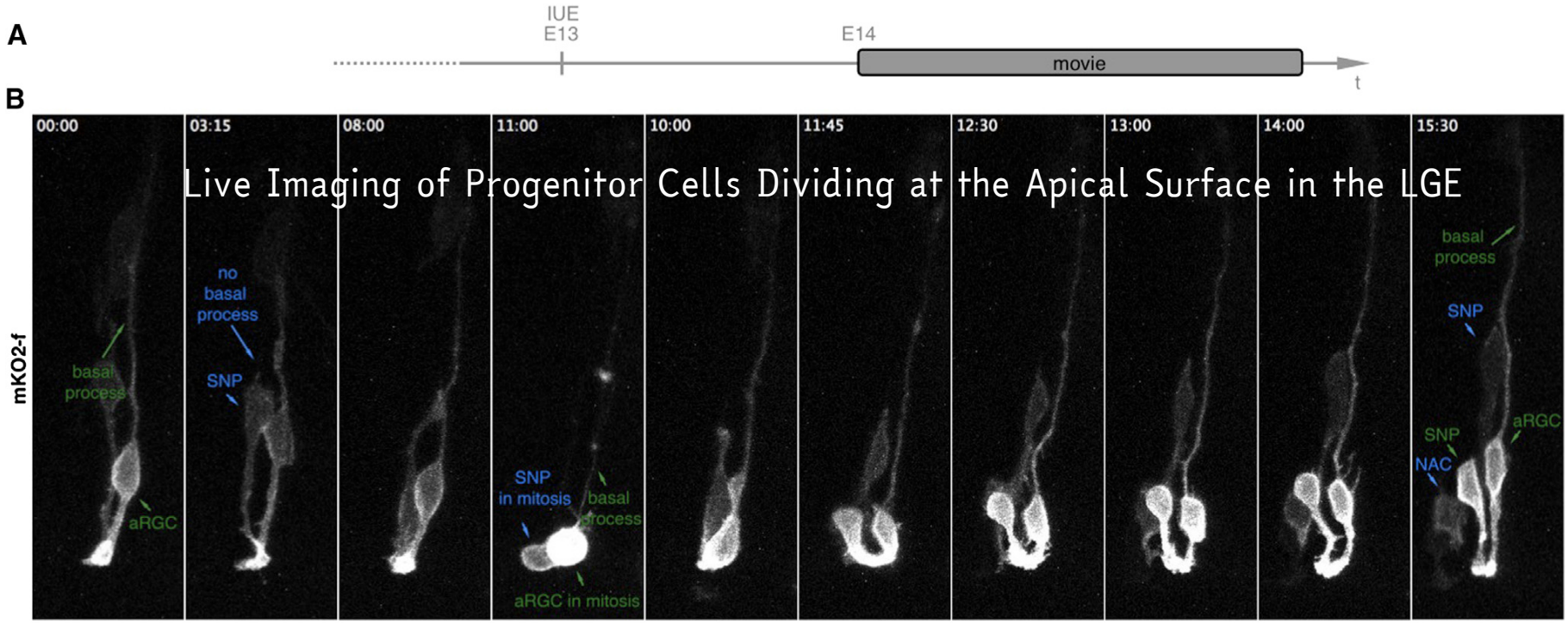
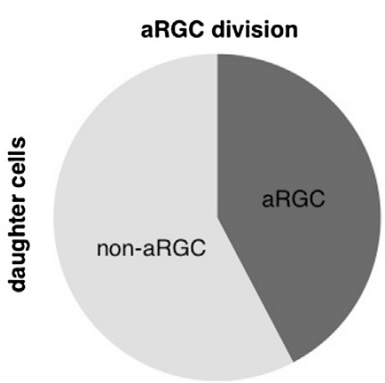
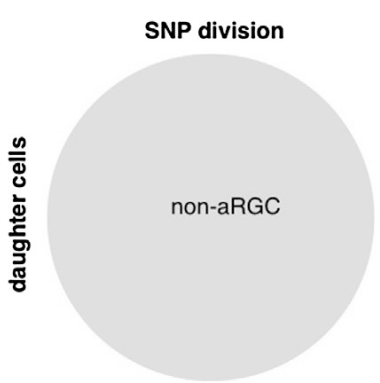


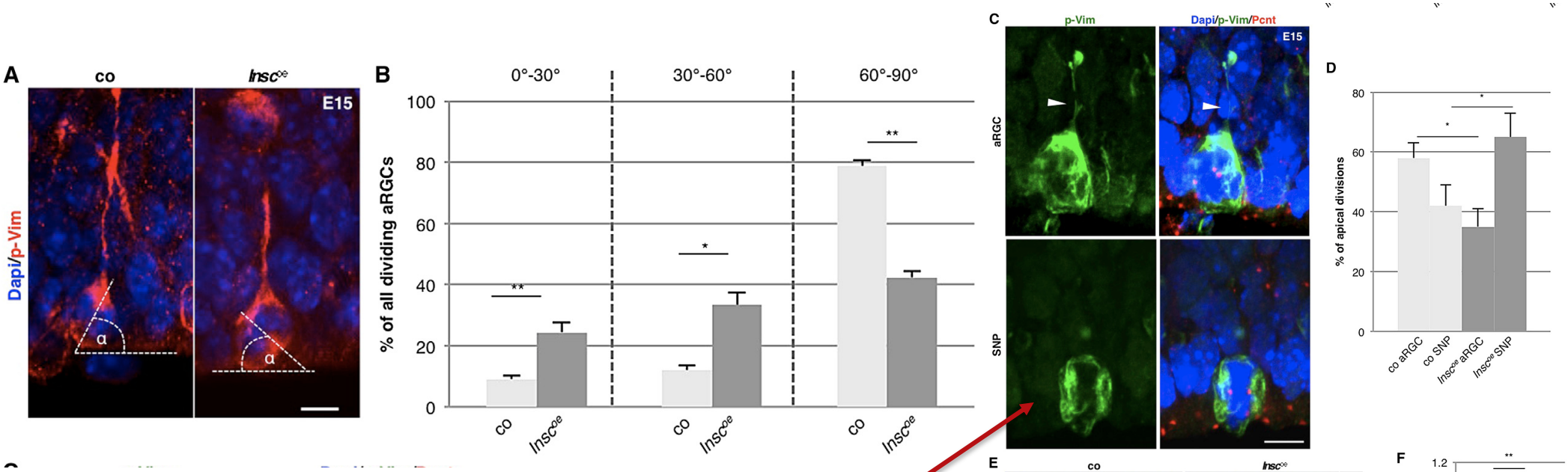
aRGCs divide almost always in an asymmetric mode





SNPs never give rise to aRGCs but generated further SNPs or different non-apical cells

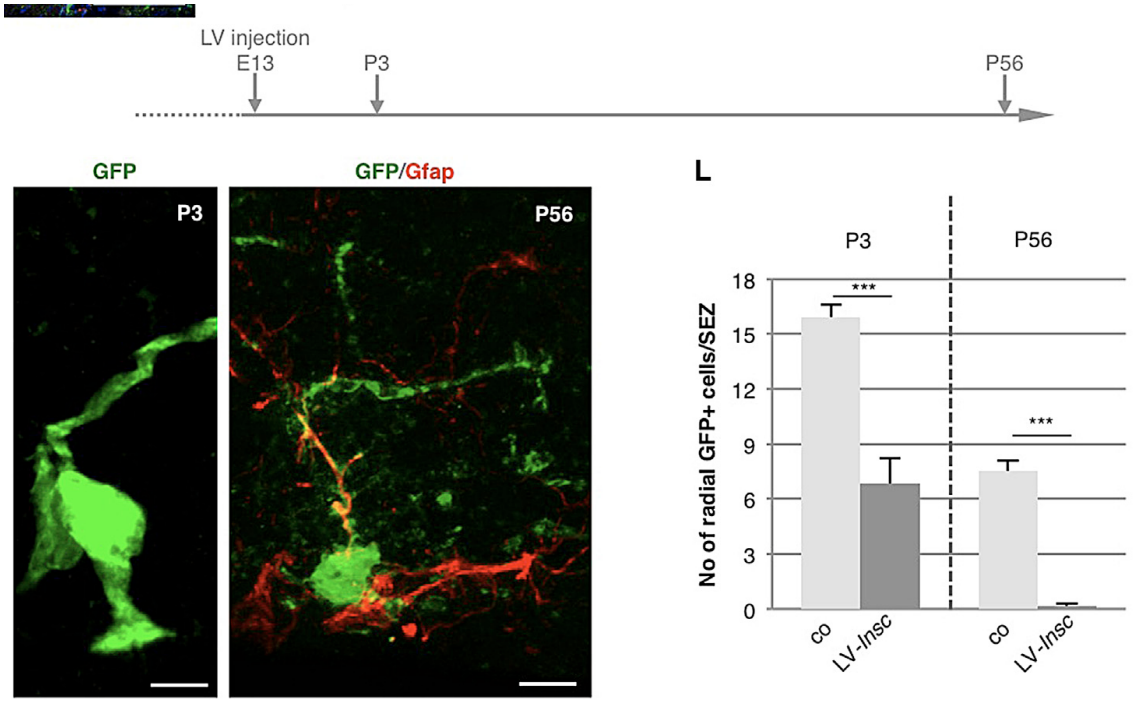
Randomizing Cleavage Plane Orientation and Depletion of aRGCs by Insc (Spindle Orientation Adaptor Protein) overexpression in the developing LGE



in Insc overexpressing mice

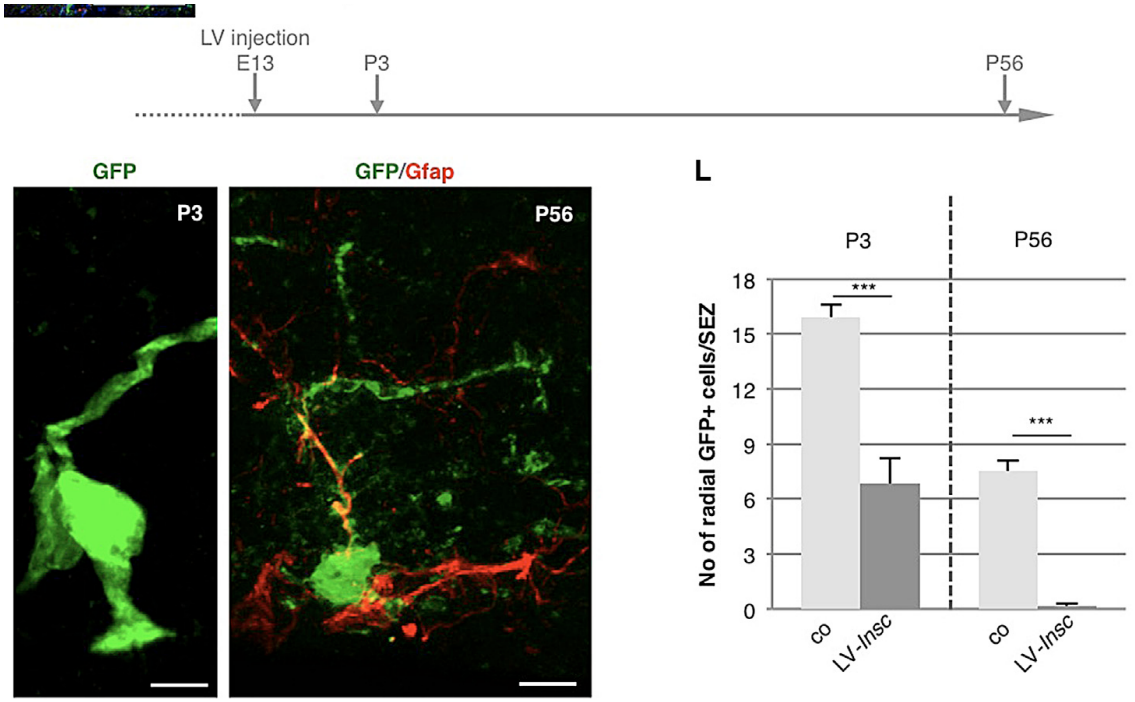
the balance in the LGE apical progenitor pool composition was altered to more SNPs

a significant decrease in the number of cells positive for p57 (inhibitor of several G1 cyclin/Cdk complexes)



Injection of a lentivirus coding for GFP (control)or Insc-IRES-GFP at E13

Quantification of the number of GFP+ cells with a radial morphology (apical endfoot at the ventricular surface and a basal process) typical for NSCs in the SEZ at P3 and adult stages (P56)



Injection of a lentivirus coding for GFP (control)or Insc-IRES-GFP at E13

Quantification of the number of GFP+ cells with a radial morphology (apical endfoot at the ventricular surface and a basal process) typical for NSCs in the SEZ at P3 and adult stages (P56)

This study shows how the cleavage plane orientation in embryonic RGCs influences the formation of adult NSCs and thereby the extent of adult neurogenesis.

The cleavage plane orientation in embryonic RGCs is a crucial factor determining the number of adult NSCs and thereby also controls the number of young adult born neurons

Cell Rep. 2019 Apr 9;27(2):429-441.e3. doi: 10.1016/j.celrep.2019.01.088.

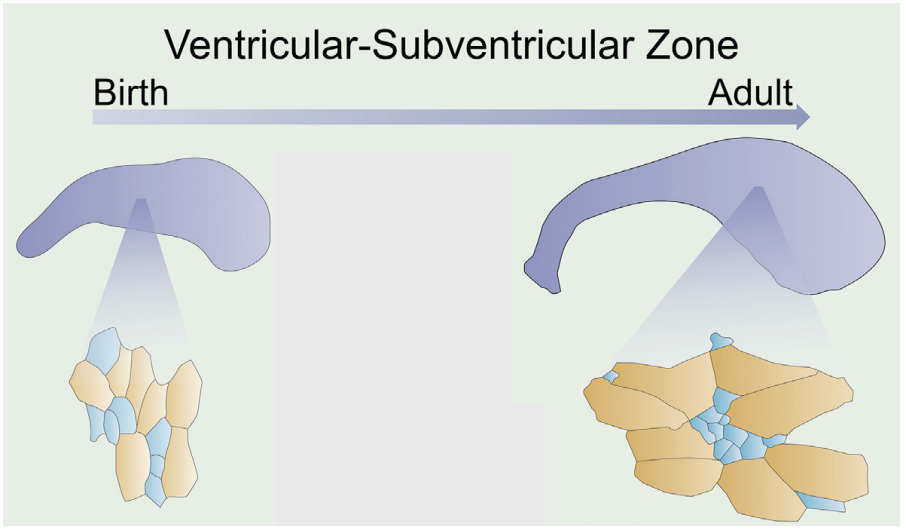
Development of Ependymal and Postnatal Neural Stem Cells and Their Origin from a Common Embryonic Progenitor.

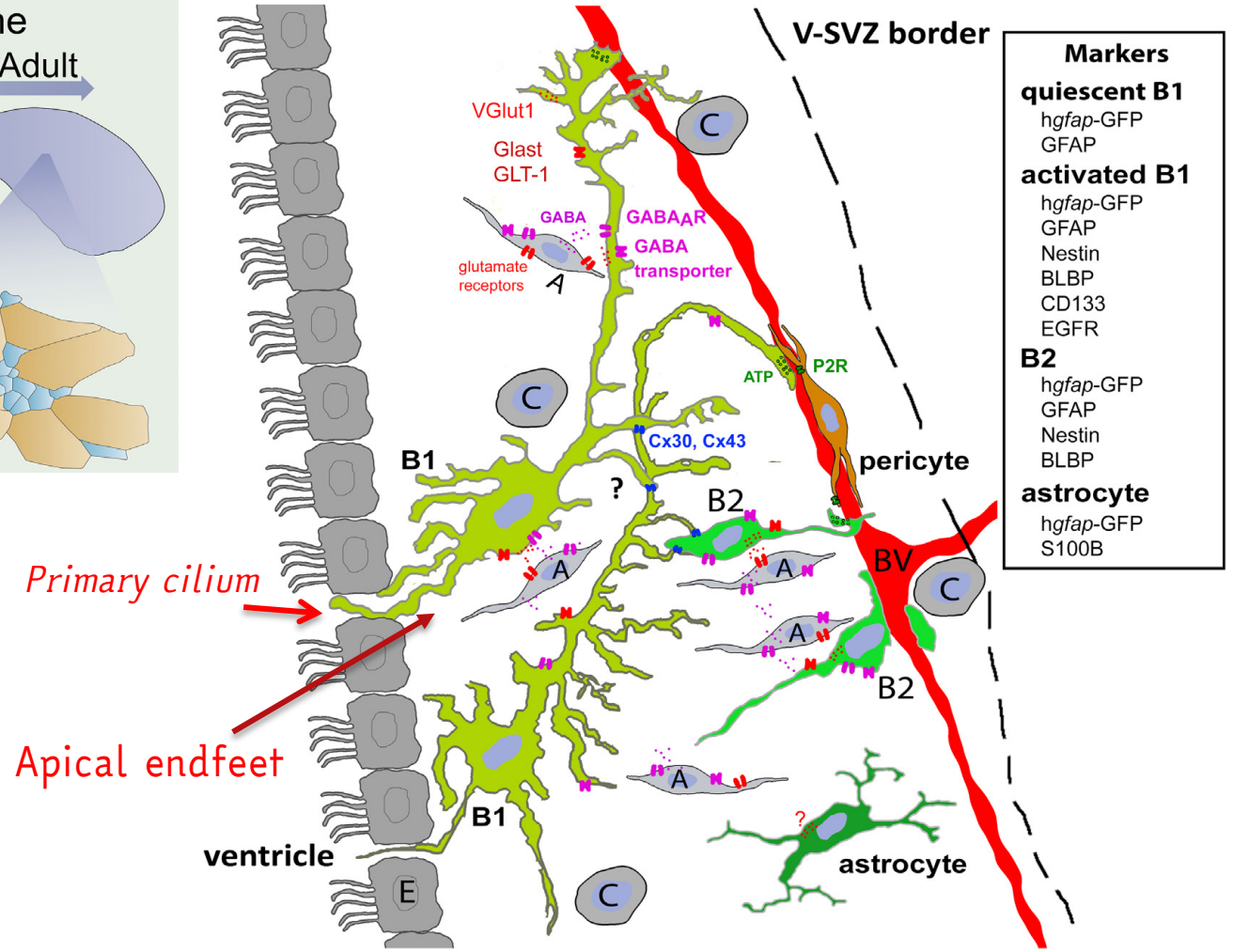
Redmond SA[1], Figueres-Oñate M[2], Obernier K[1], Nascimento MA[1], Parraguez JI[1], López-Mascaraque L[2], Fuentealba LC[1], Alvarez-Buylla A[3].

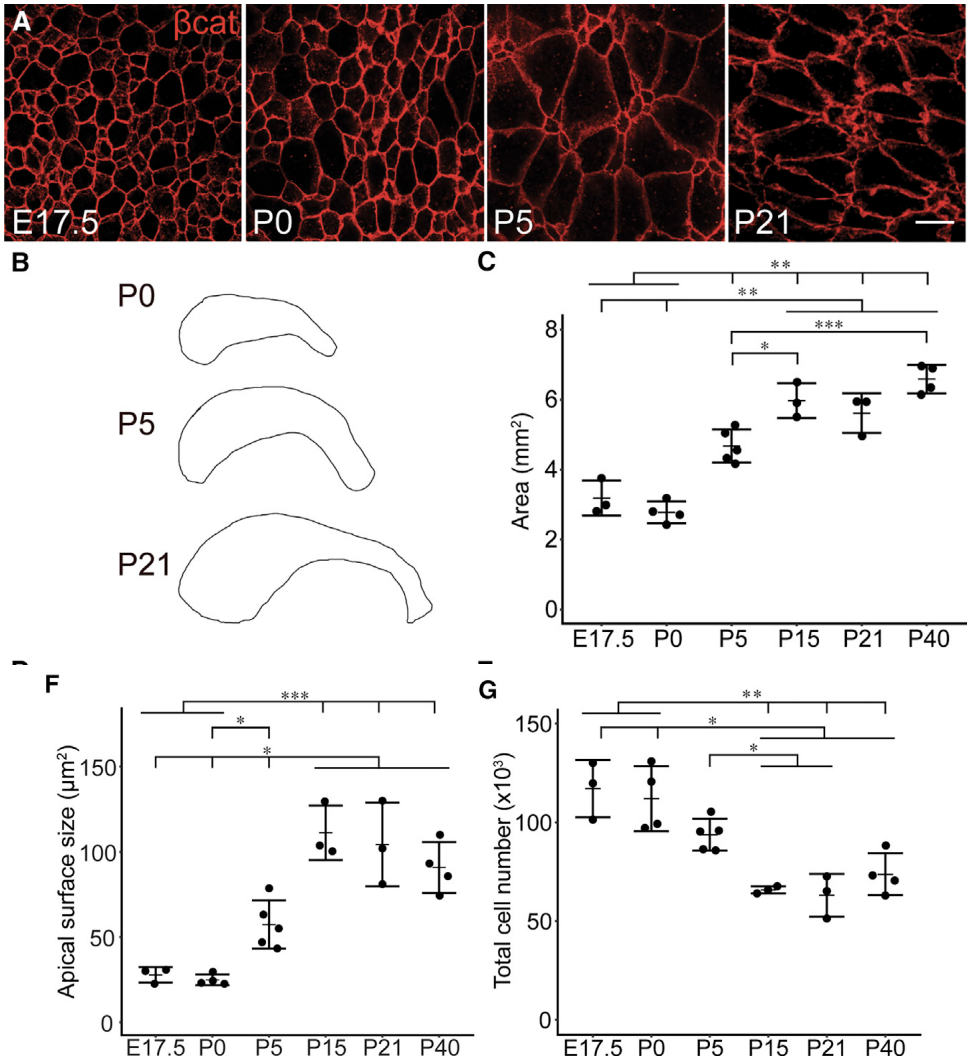
Author information

Abstract

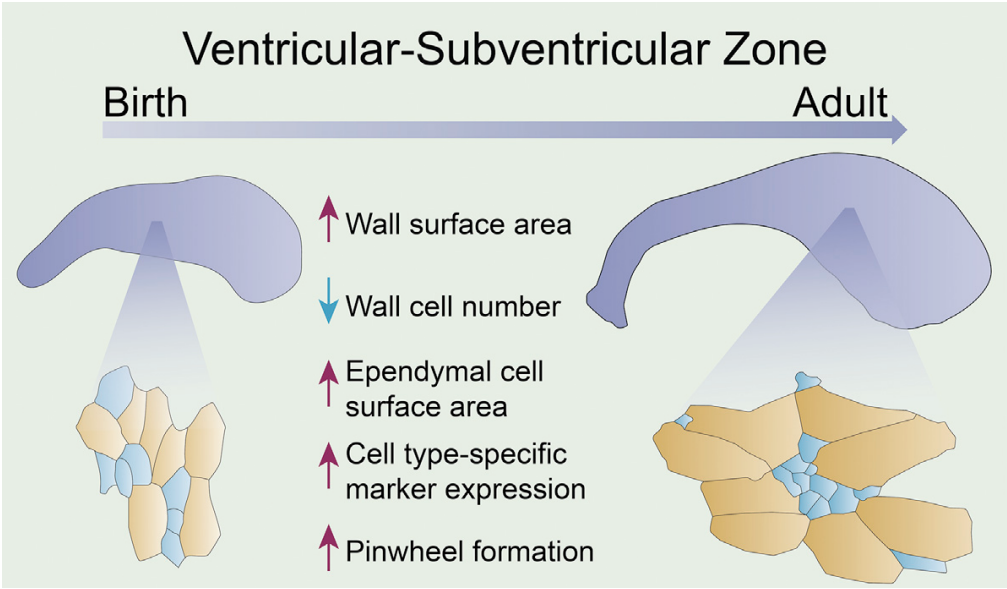
The adult mouse brain contains an extensive neurogenic niche in the lateral walls of the lateral ventricles. This epithelium, which has a unique pinwheel organization, contains multiciliated ependymal (E1) cells and neural stem cells (B1). This postnatal germinal epithelium develops from the embryonic ventricular zone, but the lineage relationship between E1 and B1 cells remains unknown. Distinct subpopulations of radial glia (RG) cells in late embryonic and early postnatal development either expand their apical domain >11-fold to form E1 cells or retain small apical domains that coalesce into the centers of pinwheels to form B1 cells. Using independent methods of lineage tracing, we show that individual RG cells can give rise to clones containing E1 and B1 cells. This study reveals key developmental steps in the formation of the postnatal germinal niche and the shared cellular origin of E1 and B1 cells.


Ventricular-Subventricular Zone
Birth
Adult


V-SVZ border
Markers
quiescent B1
hgfap-GFP
GFAP
activated B1
hgfap-GFP
GFAP
Nestin
BLBP
CD133
EGFR
B2
hgfap-GFP
GFAP
Nestin
BLBP
astrocyte
hgfap-GFP
S100B
VGlut1
Glast
GLT-1
GABA
GABA$_A$R
GABA transporter
glutamate receptors
ATP
P2R
Cx30, Cx43
pericyte
B1
B2
BV
Primary cilium
Apical endfeet
ventricle
astrocyte
C
A
E



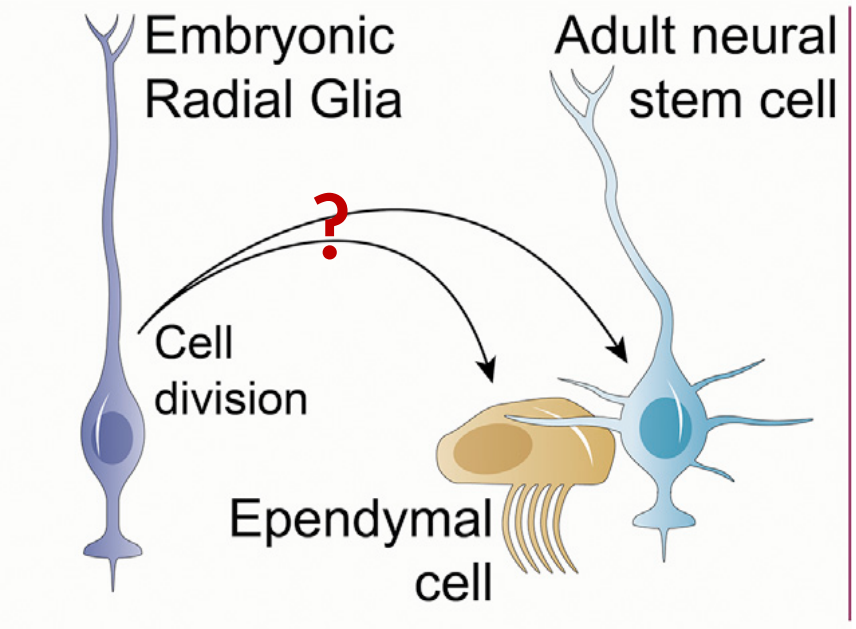
Confocal images of the surface of whole mounts of the lateral wall of the lateral ventricle at different ages. In red, b-catenin delineates the apical profiles of ventricular epithelial cells.



Redmond et al., 2019 Cell Reports

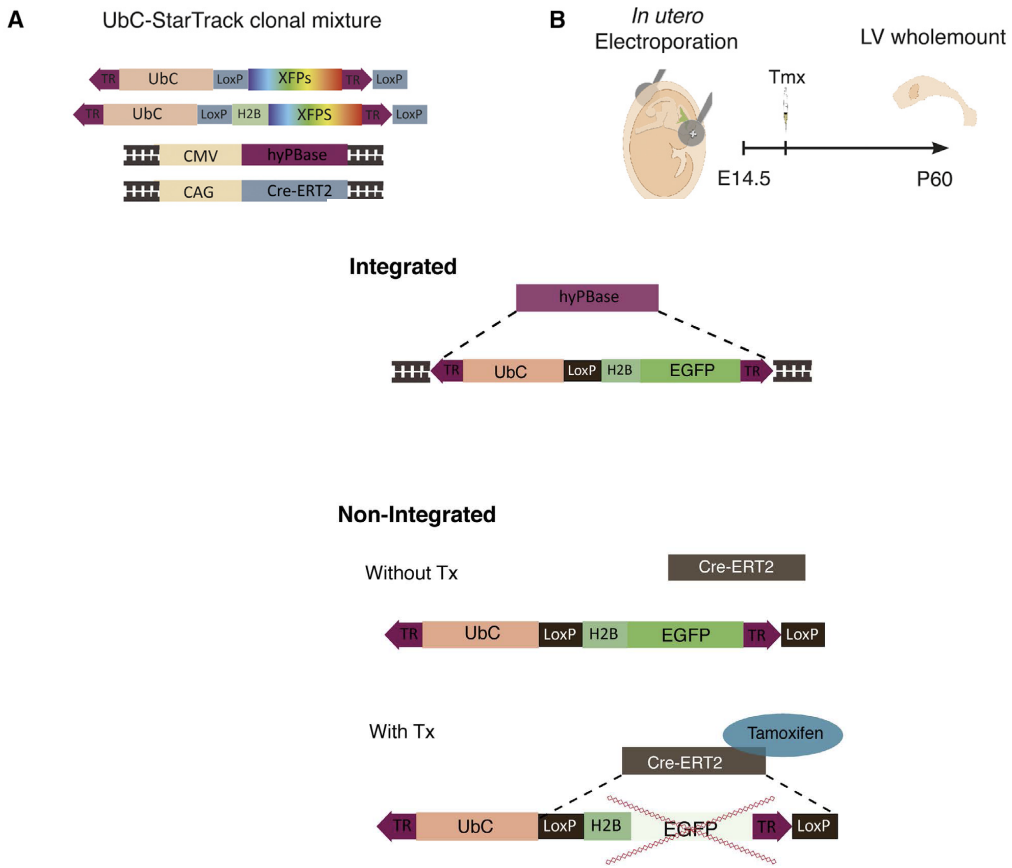
The time of birth of ependymal cells closely matches the time when RG cells give rise to B1 cells

Lineage relathionship?



Do Ependymal Cells and Postnatal NSCs Share a Common Origin?

StarTrack Lineage Tracing of Embryonic Radial Glia at E14.5



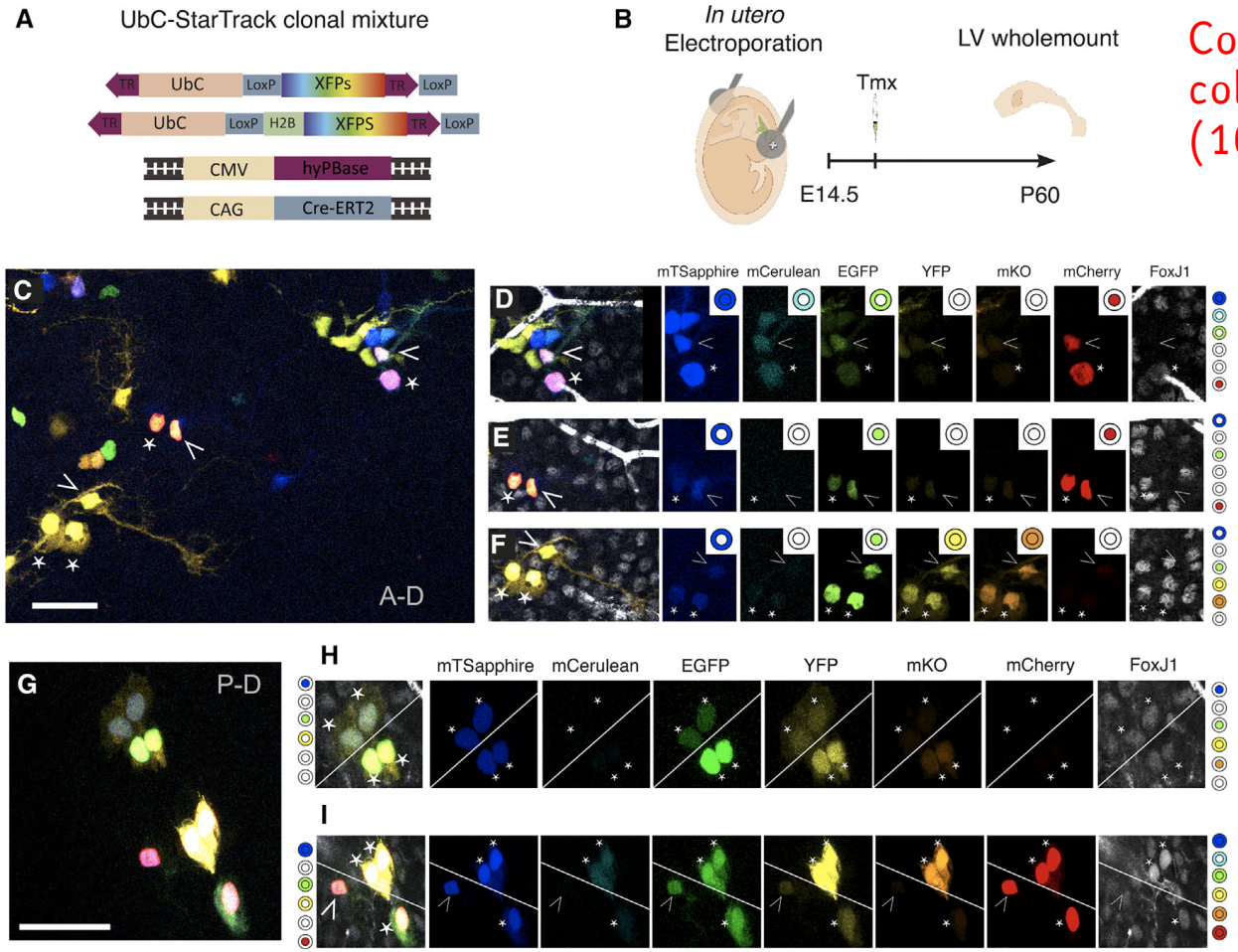
→ non-integrated constructs might gradually be diluted by successive cell divisions or may be episomally maintained, affecting the clonal code of single-cell progeny.

→ Inhibition of the potential residual episomal plasmids by using the tamoxifen (Tx) inducible Cre-Lox system.

The Cre-recombinase recognizes and cleaves the region flanked by two LoxP-sites strategically inserted into the UbC-*StarTrack* constructs.

After Tx administration, Cre cleaves the fluorescent reporter genes flanked by the LoxP-sites in the episomal copies that have not been integrated into the genome

When hyPBase driven integration does occur, one LoxP site flanking the XFP is deleted to ensure that CreERT2 does not affect the expression of the XFPs incorporated into the host genome.



Continuous expression of a heritable color code with very high complexity (16 x 10^6 possible combinations)

Time of injection	E1-E1	E1-B1	Single E1	Analyzed cells
WM#1	12	3	73	296
WM#2	6	5*	42	363
WM#3	2	0	10	185

Numbers of E1-E1, E1-B1, and single E1 clones identified by analyzing the color codes of 844 cells in three whole mounts injected and electroporated at E14.5

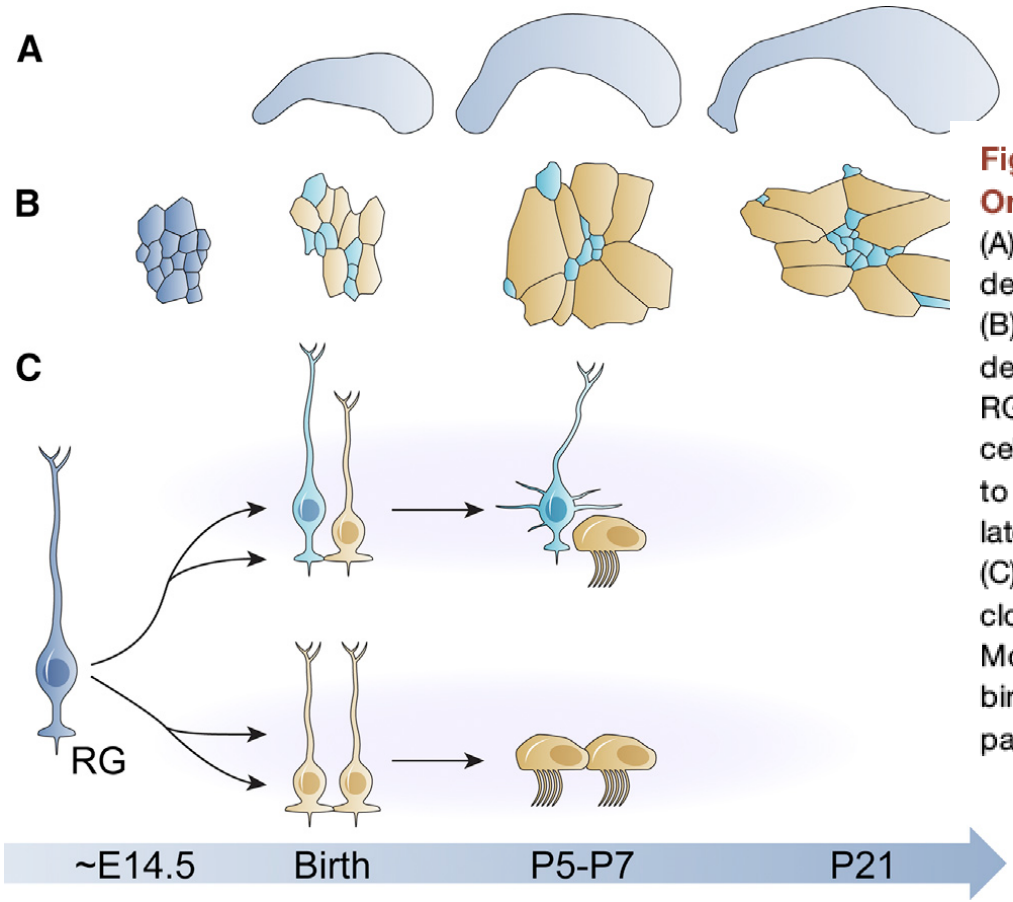
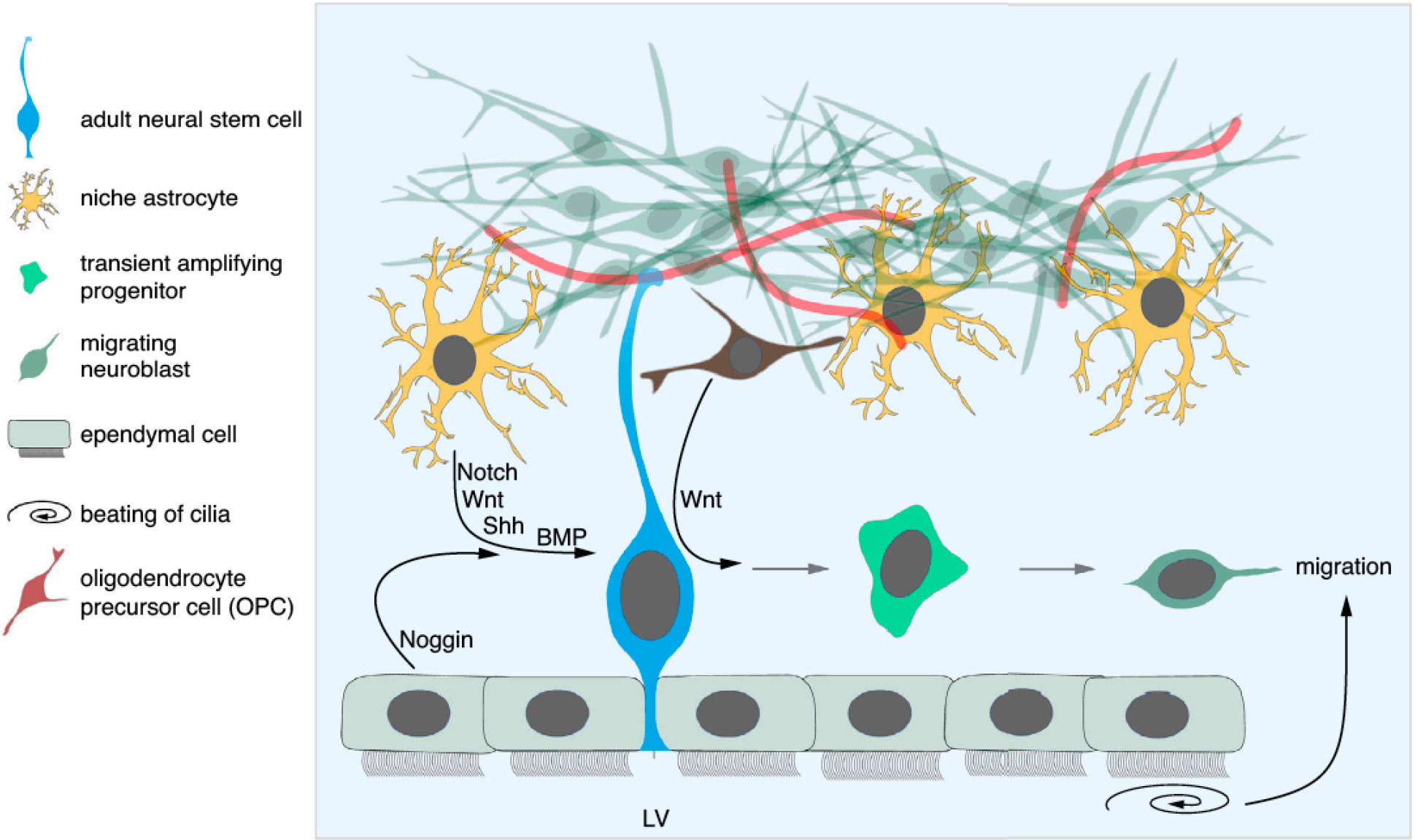


Figure 6. Summary and Proposed Model of the Developmental Origins of E1 and B1 Cells

(A) The lateral wall of the lateral ventricle nearly doubles in size after birth, despite decreasing numbers of ventricular epithelial cells.

(B) The apical surface sizes of ventricular epithelial cells increase in late fetal development and postnatally. As E1 (gold) and B1 cells (teal) differentiate from RG cells (dark blue), they start to express cell type-specific markers, and E1 cells begin to dramatically expand their apical surfaces. By P5, pinwheels start to form in the P-V region of the whole mount and are found in all regions of the lateral wall after P10.

(C) An embryonic RG cell (left) divides around E14.5 to give rise to two types of clones: mixed E1-B1 clones (top row) or E1-E1 clones (bottom row). Morphological and molecular maturation of E1 and B1 cells begins around birth and is largely completed by P7. The age associated with each cartoon panel in (A)–(C) is located on the blue timeline directly below the image.



Falk and Gotz 2017 Current Opinion in Neurobiology

Cell

Article

Embryonic Origin of Postnatal Neural Stem Cells

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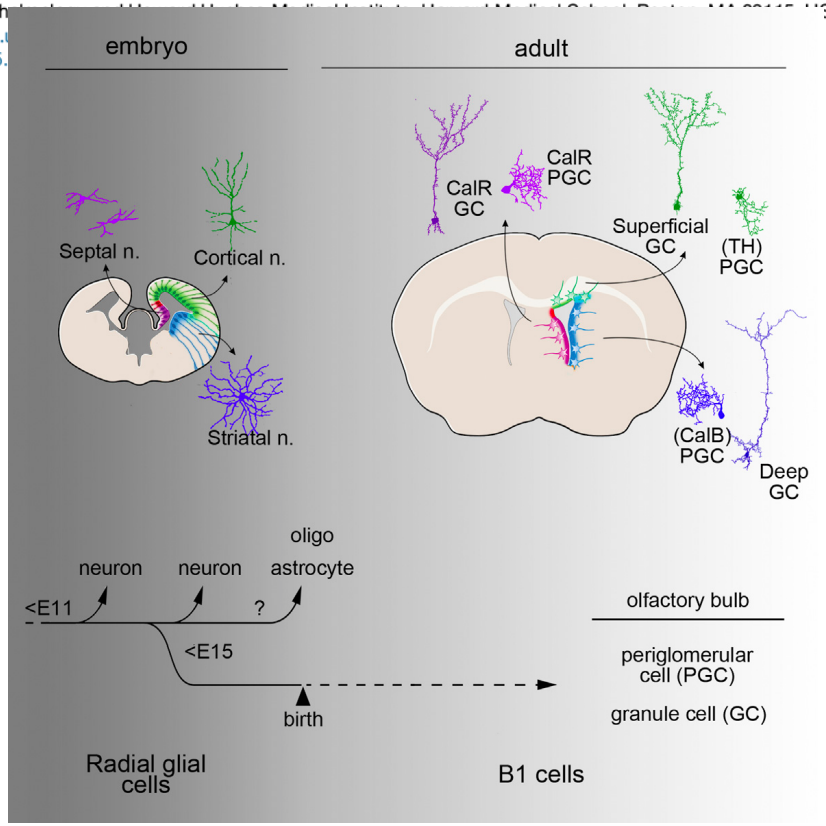
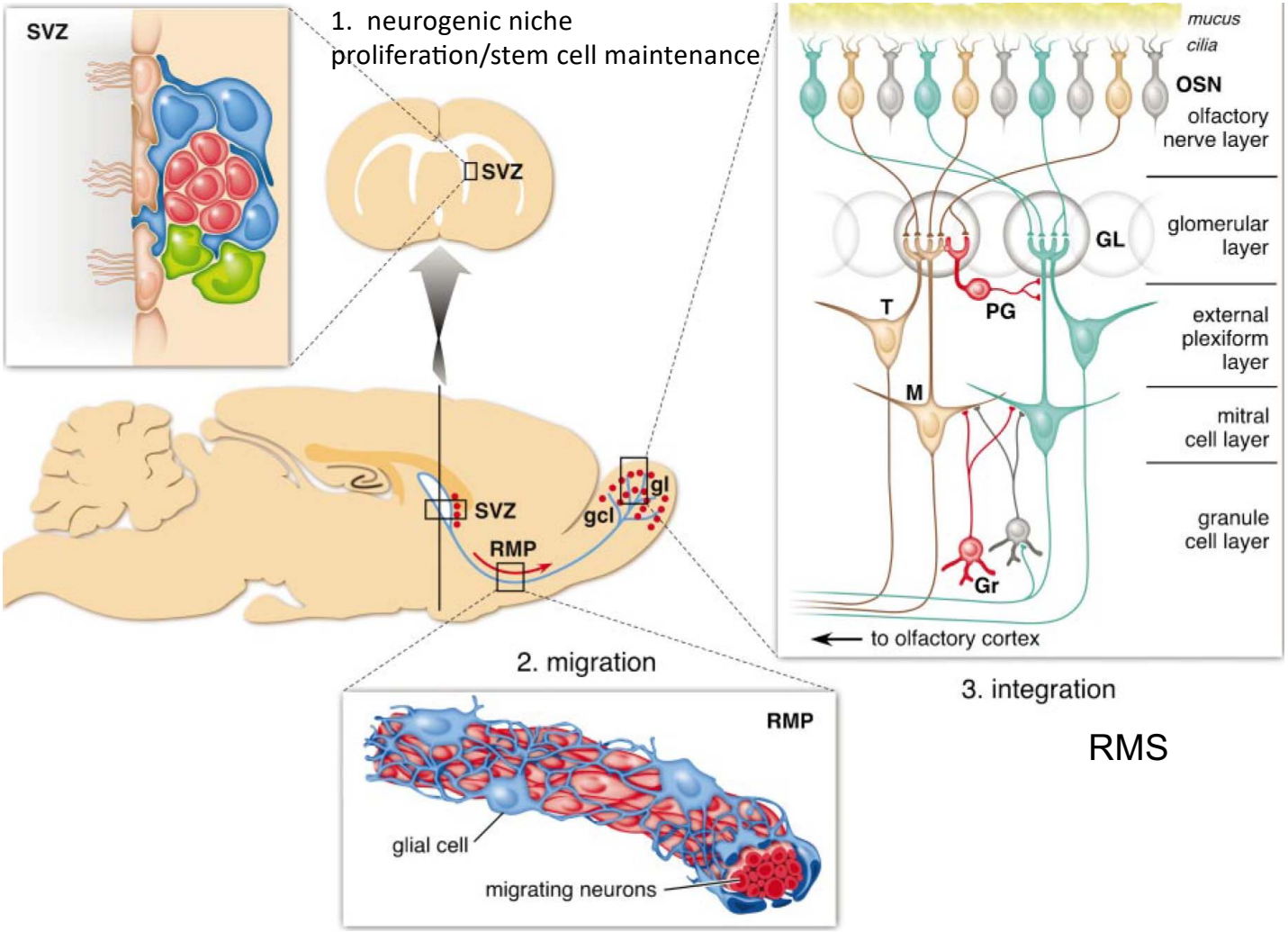


Here we show that the majority of mouse B1 cell precursors are produced between embryonic days (E) 13.5 and 15.5 and remain largely quiescent until they become reactivated postnatally.

Using a retroviral library carrying over 100,000 genetic tags, we found that B1 cells share a common progenitor with embryonic cells of the cortex, striatum, and septum, but this lineage relationship is lost before E15.5.

The regional specification of B1 cells is evident as early as E11.5 and is spatially linked to the production of neurons that populate different areas of the forebrain.

This study reveals an early embryonic regional specification of postnatal neural stem cells and the lineage relationship between them and embryonic progenitor cells.

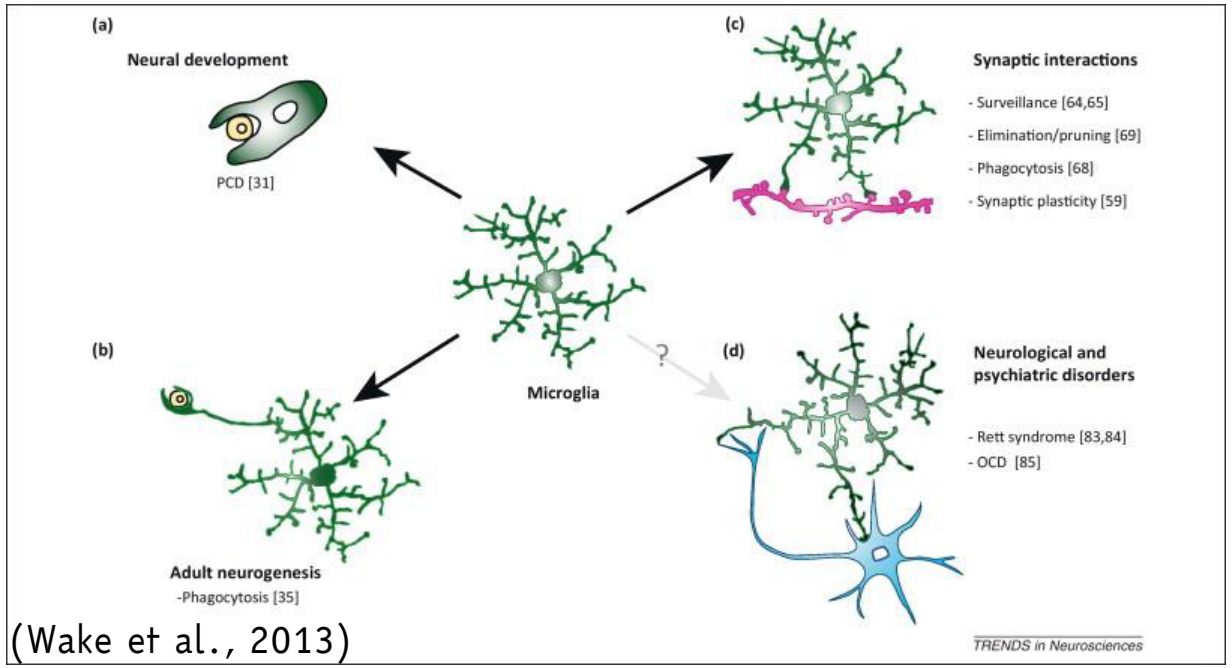

SVZ
1. neurogenic niche
proliferation/stem cell maintenance
SVZ
SVZ
gcl
gl
RMP
2. migration
RMP
glial cell
migrating neurons
mucus
cilia
OSN
olfactory
nerve layer
GL
glomerular
layer
T
PG
external
plexiform
layer
M
mitral
cell layer
granule
cell layer
Gr
to olfactory cortex
3. integration

RMS

Microglia = Immune effector cells of the CNS

(approximately 10-20% of the total population of glial cells in the adult brain)

Derived from blood-borne macrophages, which migrate into the CNS during development



(Wake et al., 2013)