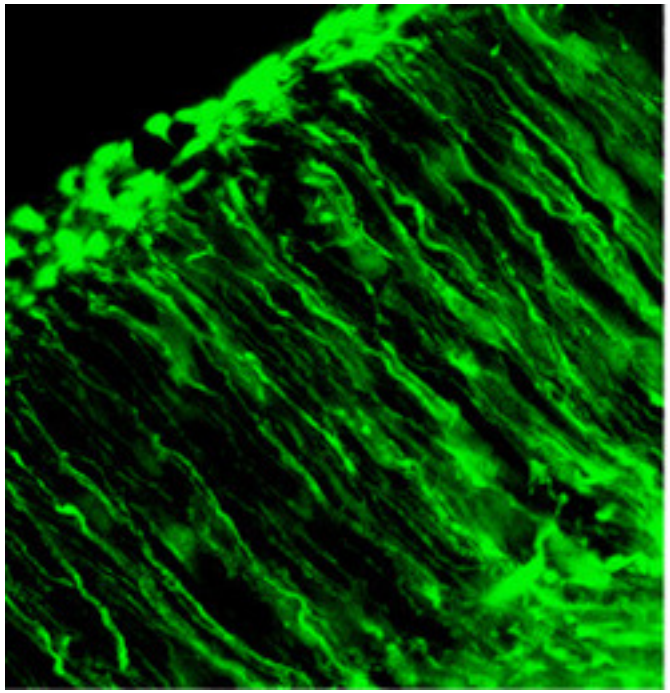
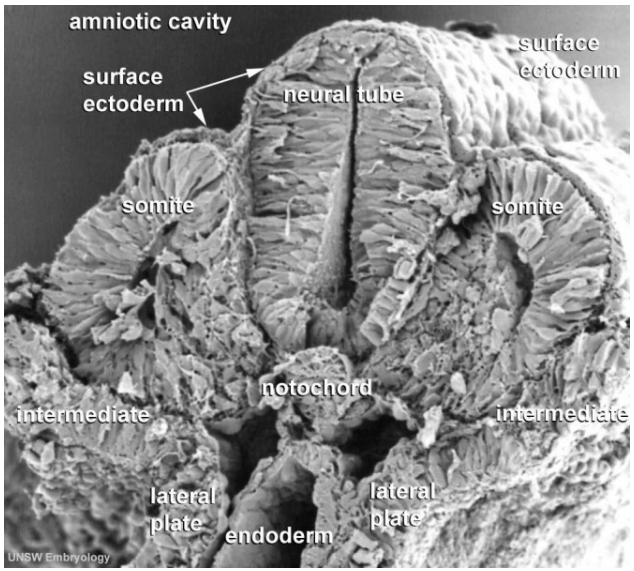
Radial glial cells
key organizers in CNS development



Radial glia

Neural progenitors



Neuroepithelial cells

- ✓ The CNS forms from a pseudostratified neuroepithelium lining the fluid-filled ventricle of the neural tube.
- ✓ This epithelium is composed by **proliferative neuroepithelial cells** which maintain apico-basal polarity by contacting both the ventricular and pial surface.
- ✓ During development, **neural stem cells** give rise to all the neurons and glial cells of the mammalian central nervous system
- ✓ For the most part, neurogenesis precedes gliogenesis

neurogenesis

gliogenesis

Developing chick spinal cord

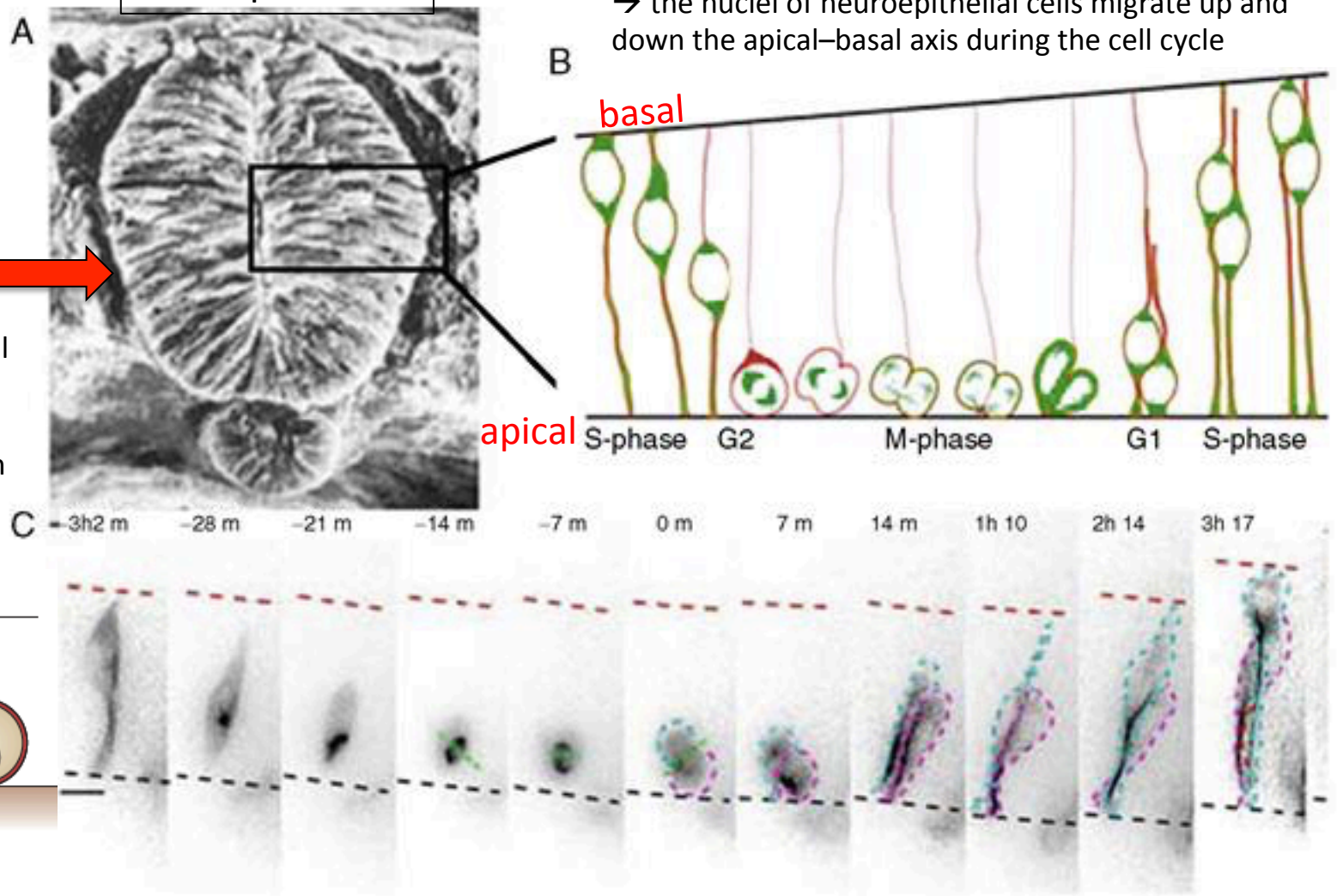
Interkinetic nuclear migration

→ the nuclei of neuroepithelial cells migrate up and down the apical–basal axis during the cell cycle

polarised pseudostratified neuroepithelium

The basal side is attached to the pial surface

The apical side contacts the lumen of the neural tube



Self-renewing **neuroepithelial cells** line the ventricles throughout the neuraxis at the stages of neural tube closure – **neuroepithelial cells are the major neuronal progenitors in the spinal cord (symmetric vs asymmetric divisions)**

→ two general criteria applied to define **STEM CELLS**

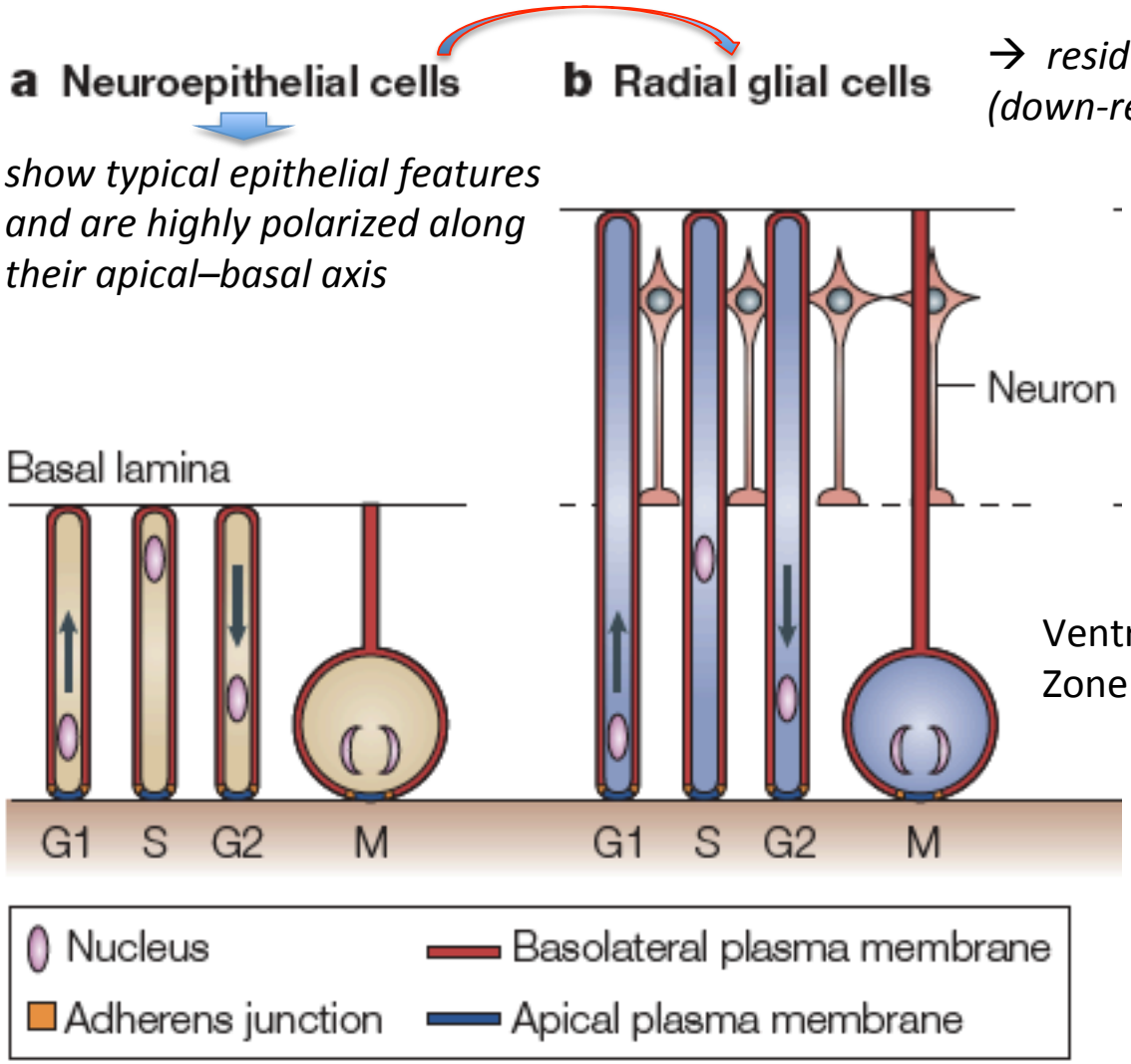
1) **self-renewal**, ideally for an unlimited number of cell divisions, and
2) **multipotency** (the ability to give rise to numerous types of differentiated cell)

BUT in the CNS → the term stem cells describes neural cells that are:

1) self-renewing, not necessarily for an unlimited number of cell divisions, and
2) might be multipotent or unipotent

→Neural stem cells/progenitors

After the onset of neurogenesis, neuroepithelial cells give rise to a distinct, but related, cell type: **radial glial cells**



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

show typical epithelial features and are highly polarized along their apical–basal axis

Most of the neurons in the brain are derived (directly or indirectly) from radial glial cells

The transmembrane protein prominin-1 (CD133) is selectively found in the apical plasma membrane

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

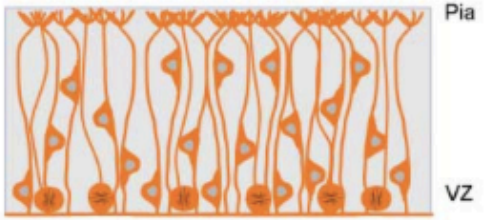
*The antiproliferative gene *Tis21* is a molecular marker that is selectively expressed in virtually all neuroepithelial cells that are about to undergo a neurogenic division, but not in proliferating neuroepithelial cells[74].

Developmental transition: from self-renewing neuroepithelial cells → to radial glial cells

Cerebral cortex

A

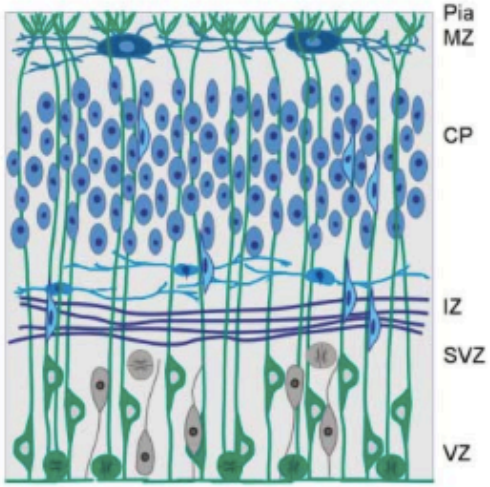
Prior to neurogenesis



Neuroepithelial cells

mid-neurogenesis

B



Radial glial cells

Neuroepithelial cells and radial glia
→ same morphology
→ can be discriminated by several antigens

Radial glia identity is promoted by Notch1 signaling in the murine forebrain (Gaiano et al., 2000….Imayoshi et al., 2010)

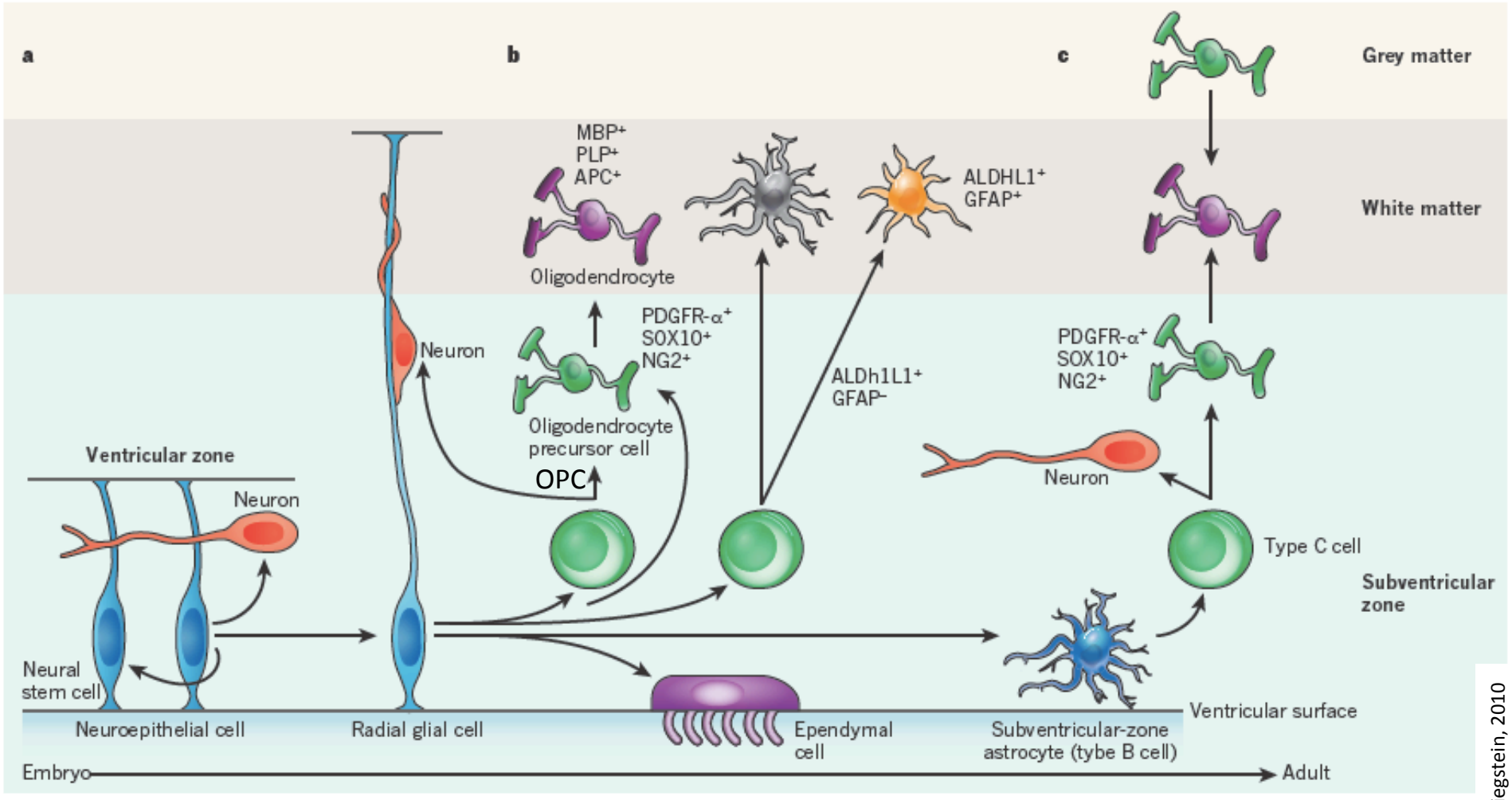
C

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

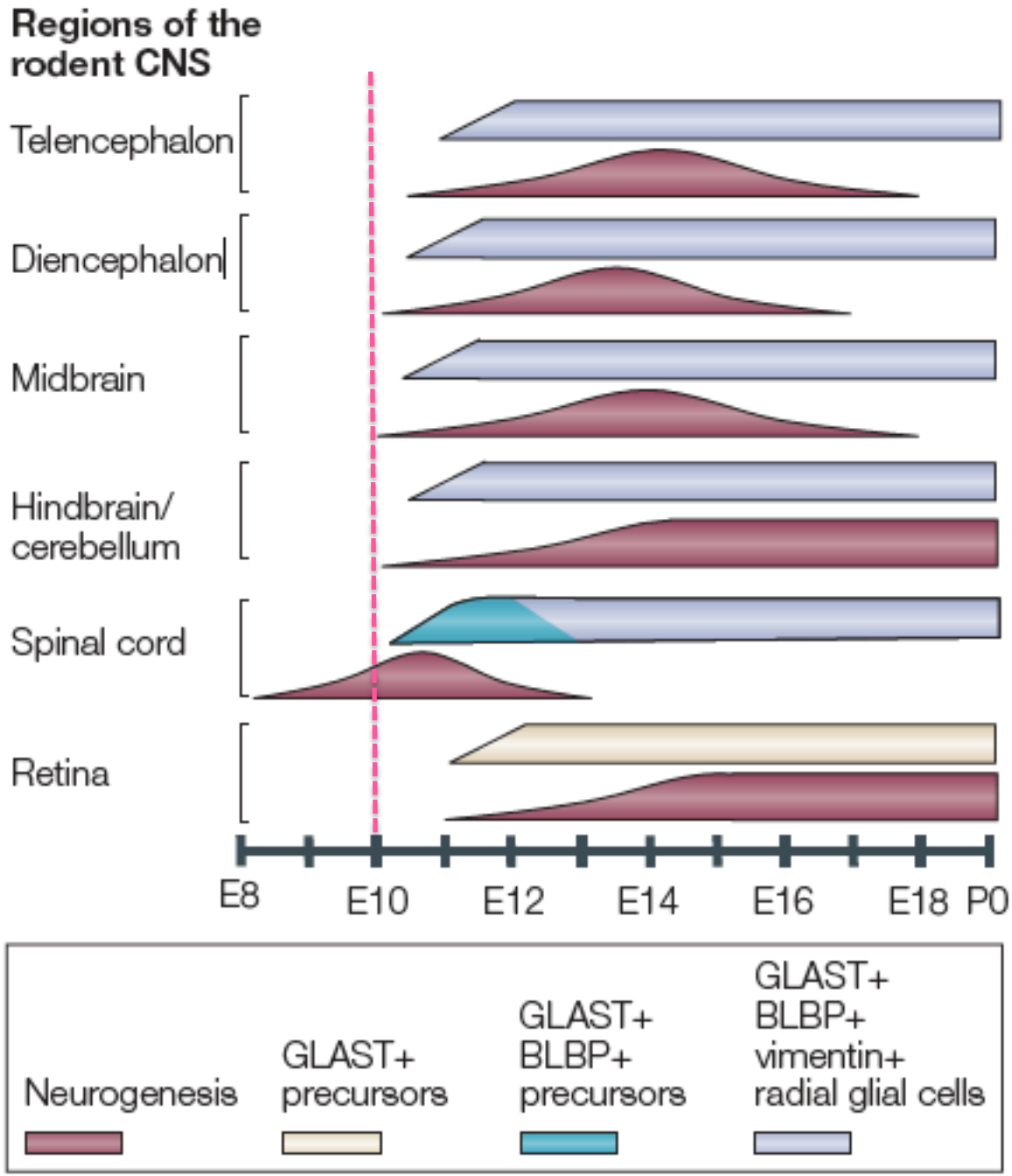
In mice, neuroepithelial – radial glia transition occurs throughout most of the brain between embryonic day 10 (E10), when no astroglial markers can yet be detected, and E12, when most CNS regions are dominated by progenitor cells that are expressing several of these astroglial features



Rowitch & Kriegstein, 2010

Our understanding of the unique role **radial glial cells** play has significantly expanded in the last decade.

Self-renewing neuroepithelial cells line the ventricles throughout the neuraxis at the stages of neural tube closure. These cells may generate some neurons. Neuroepithelial cells are transformed into radial glial cells as neurogenesis begins.

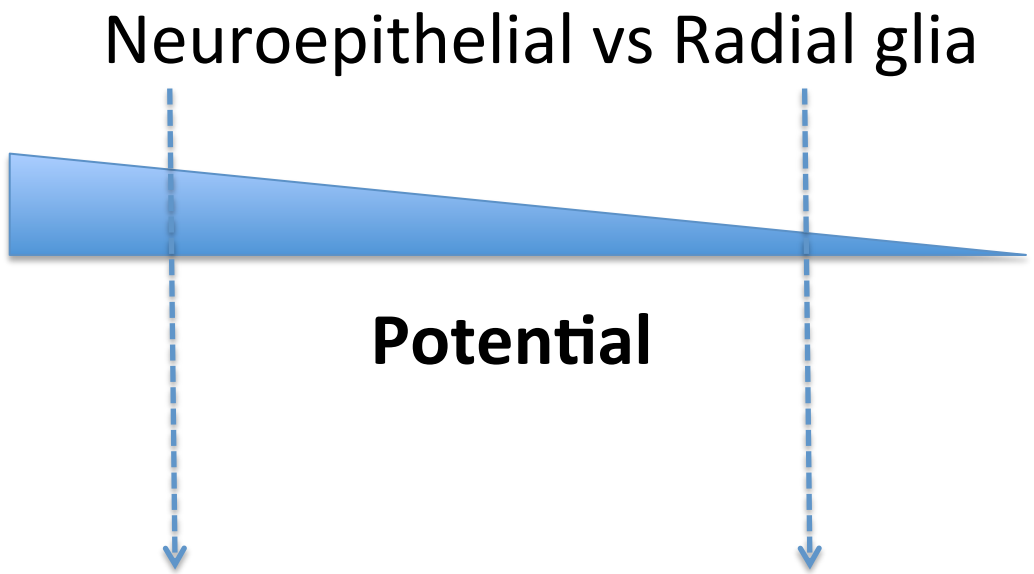


Progressive waves of **neurogenesis** begin at about E 9–10 in mice

neurogenic gradients in the brain

caudal-rostral,
ventral–dorsal
lateral–medial

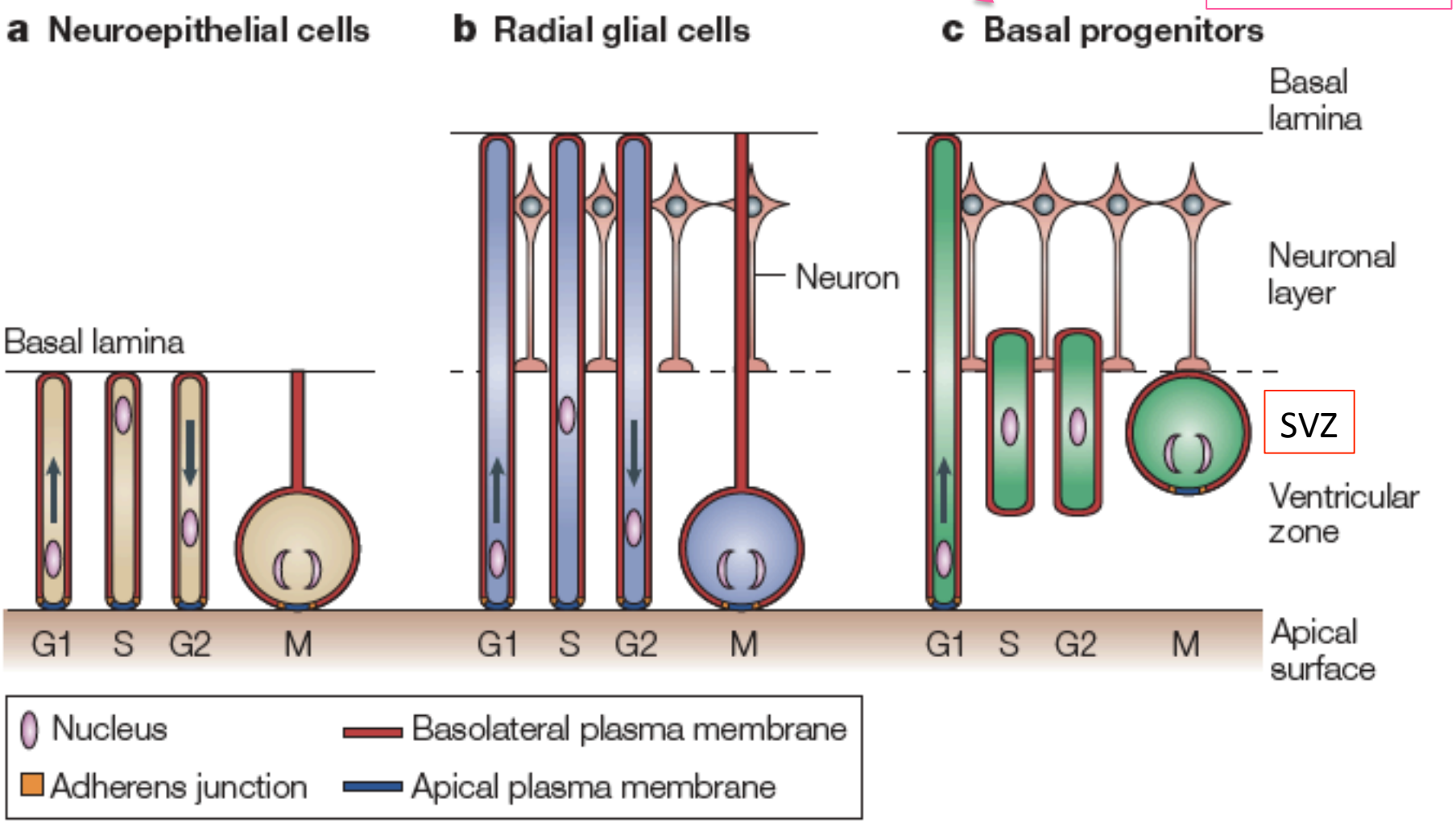
Transgenic mouse Cre-Lox recombination (*__*cell type specific promoter*__ Cre-line X Reporter line)
Replication defective Retroviral vectors carrying reporter genes (i.e. GFP)



*nestin, BLBP, GFAP

At later stages of neurogenesis, basal progenitors form the subventricular zone

Tbr2 , Cux1, Cux2



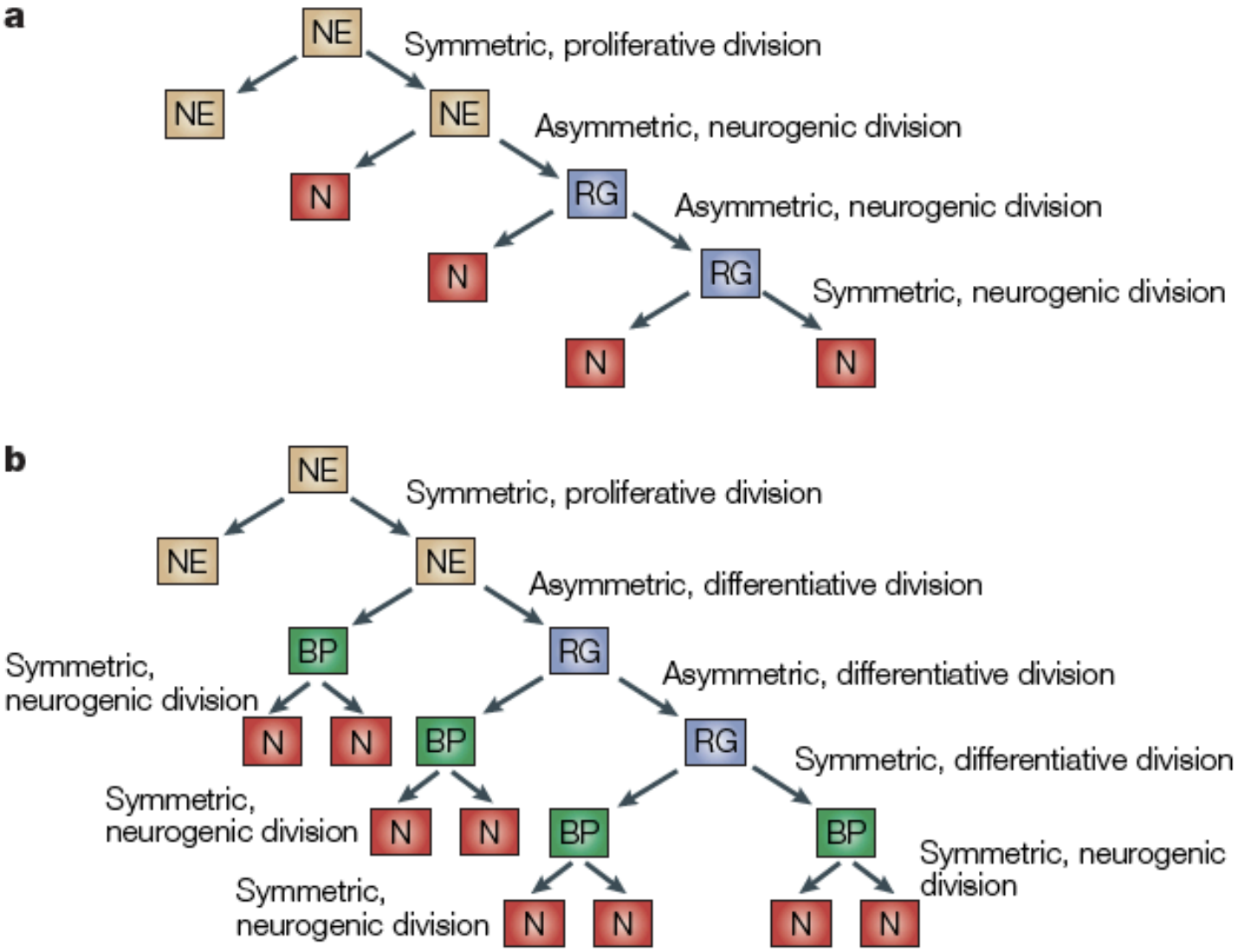


Figure 1 | **Lineage trees of neurogenesis.** The lineage trees shown provide a simplified view of the relationship between neuroepithelial cells (NE), radial glial cells (RG) and neurons (N), without (**a**) and with (**b**) basal progenitors (BP) as cellular intermediates in the generation of neurons. They also show the types of cell division involved.

The concept of dualism between neuronal and glial lineages: a historical perspective

- ✓ co-existence of **neuroblasts** and **spongioblasts**, based on the position of their soma (His, 1889)

- ✓ **neuroblasts** and **spongioblasts** are simply cells in different phases of the cell cycle (interkynetic nuclear migration) ('60)

…but still **the concept of progenitor diversity** was so well accepted that the neuroblast and spongioblast terms remain

- Pasko Rakic conied the term **Radial glial cells** (P. Rakic, 1972) in the cerebral cortex

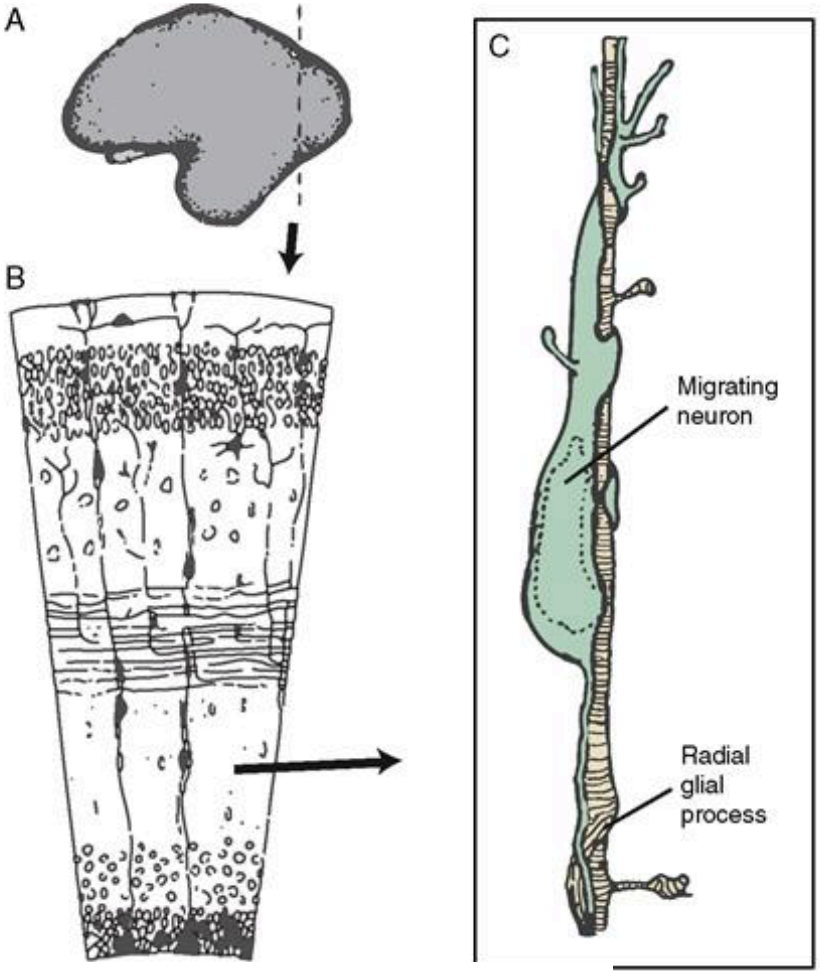
- Glial fibrillary acidic protein (GFAP) was discovered as a new 'marker' for astrocytes and was also detected **in radial glial cells** during development (1980) this supported their **glial nature** and thus implicitly **their identity as the glial progenitors**.

- 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), further suggesting the concept of dualism between neuronal and glial lineages, **implying that neuroblasts and radial glia were as distinct as neurons and astrocytes in the mature CNS.**

From Malatesta & Gotz, 2013

Pasko Rakic 1972

The “classical role” of **radial glia** is in **neuronal migration** (Rakic, 1972)



Cerebral cortex development

Radial glial cells → substrate for neuronal migration (radial)

- **Express many hallmarks of glial (astroglial) cells**

-gap-junction mediated contact with blood vessels
-contain glycogen granules
-expression of glial markers:Vimentin -GFAP (Glial fibrillary acidic protein)(*in primate cortex-not rodent cortex*)- Glast (astrocyte-specific glutamate transporter)- BLBP (Brain lipid-binding protein)

- **Share some features with neuroepithelial cells**

-display an apical-basal polarity
-express **Nestin** (and its post-translationally modified isoform RC2)

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DEV1619

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

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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

Experimental paradigm

Two independent techniques to fluorescently label radial glial cells and isolate them by FACS

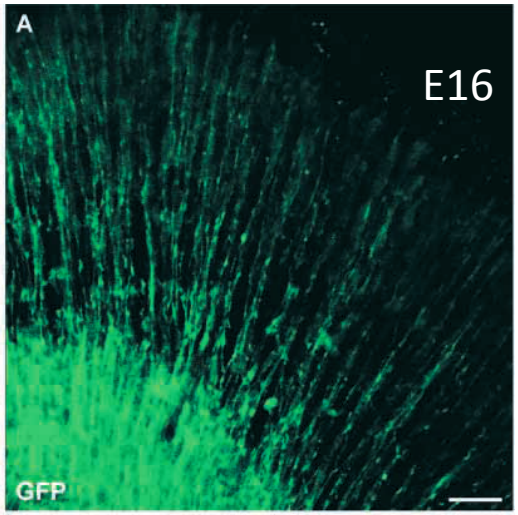
transgenic mouse line (hGFAP-GFP)

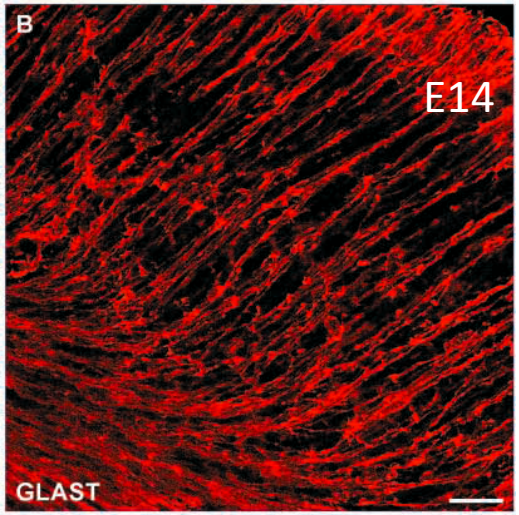
Labelling precursor cells with long radial processes with fluorescent dyes from the pial surface

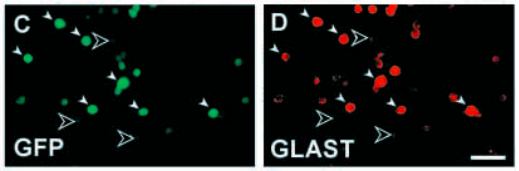
Sorted cells are cultured in vitro for 7 days and their progeny determined by the use of cell-type specific antibodies
anti-b-tubulin-III → neurons
anti-GFAP → astrocytes
anti-O4→ oligodendrocyte precursors

?

How to determine the potential of single clones?

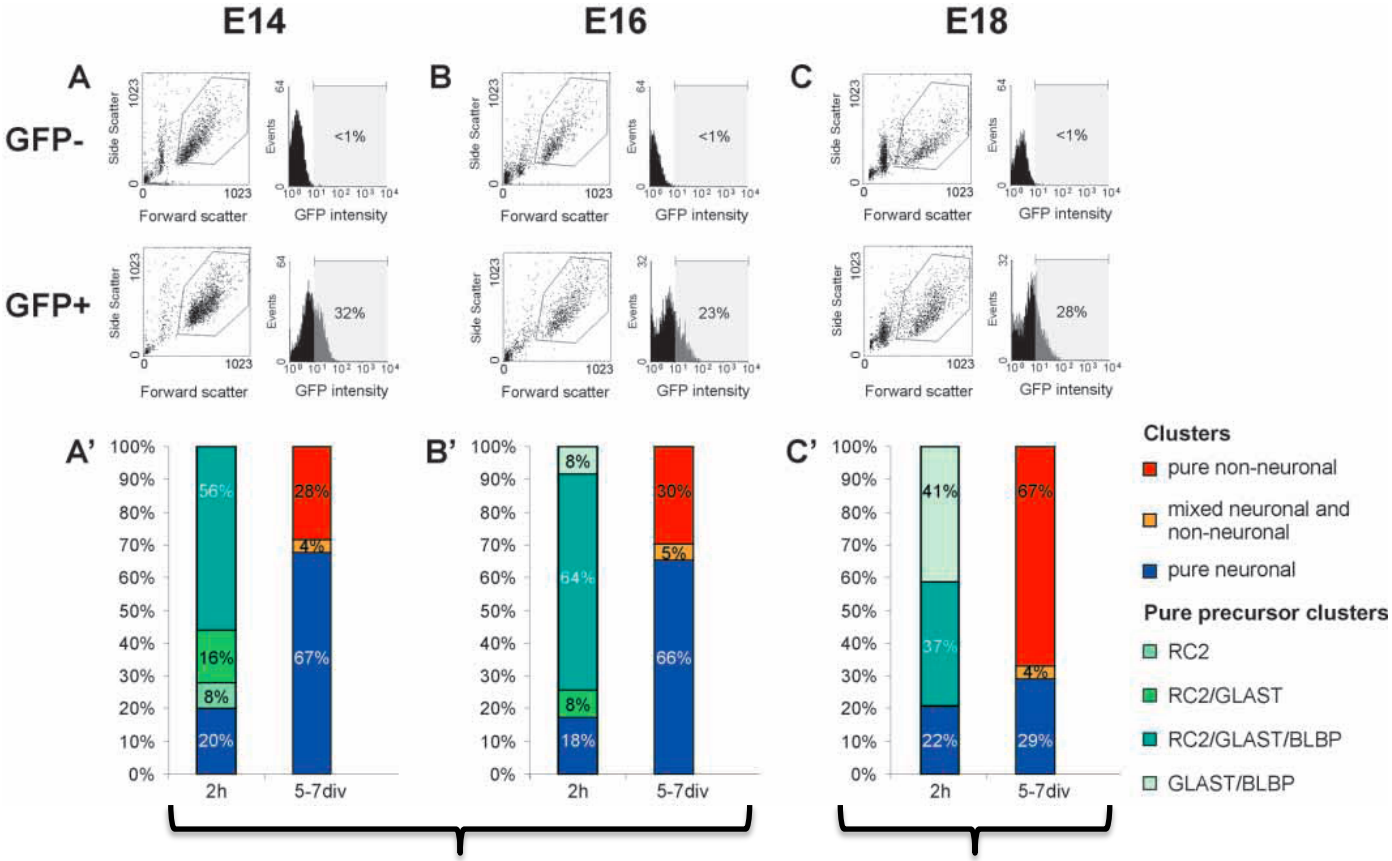






GFP-immunostained cells from acutely dissociated E14 cortex double stained with GLAST antiserum

Characterization of green fluorescent cells isolated from the cortex of hGFAP-GFP transgenic mice and their progeny



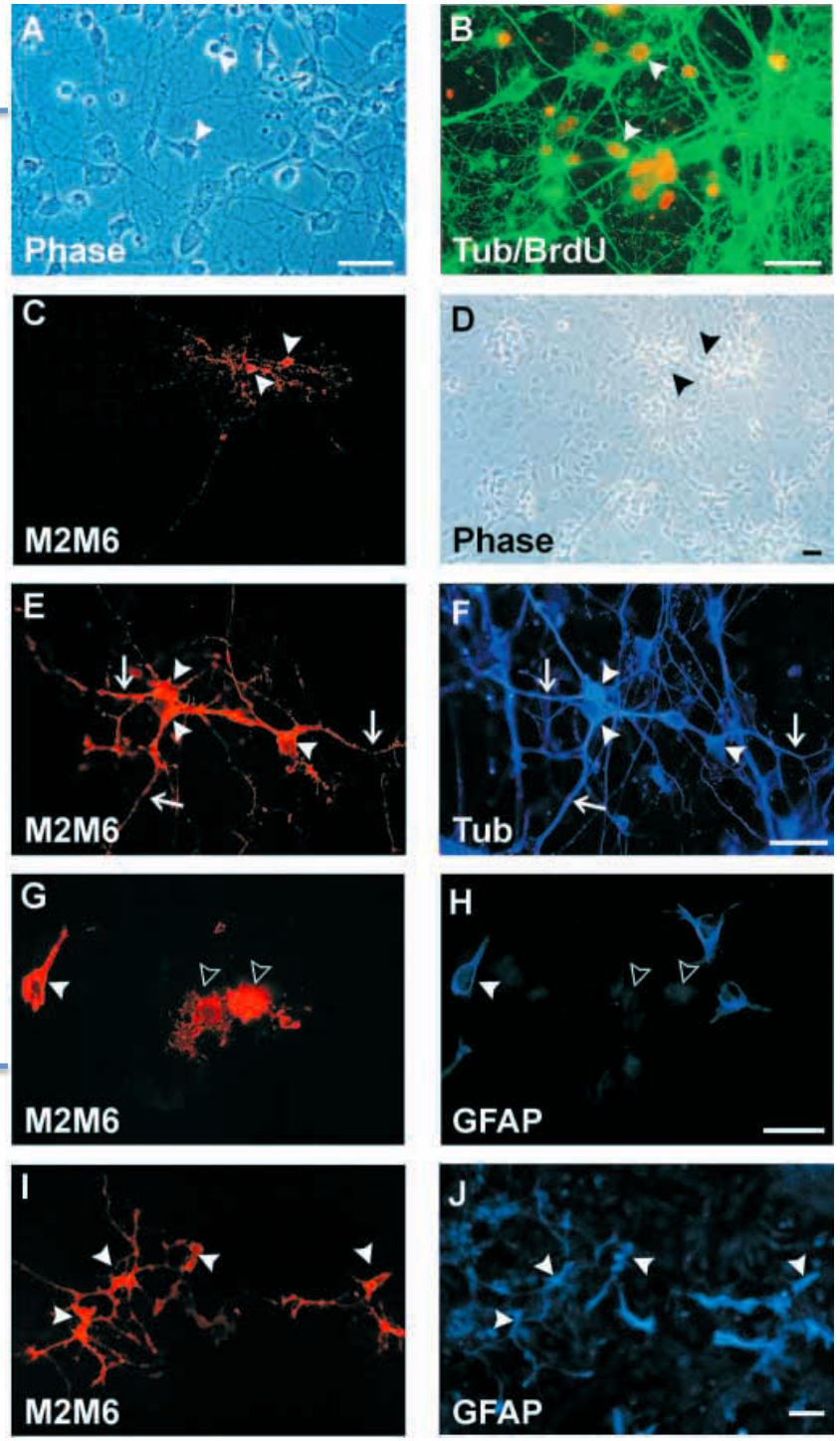
the predominant cell type observed after 1 week in vitro was neuronal

the predominant cell type observed after 1 week in vitro was glia

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

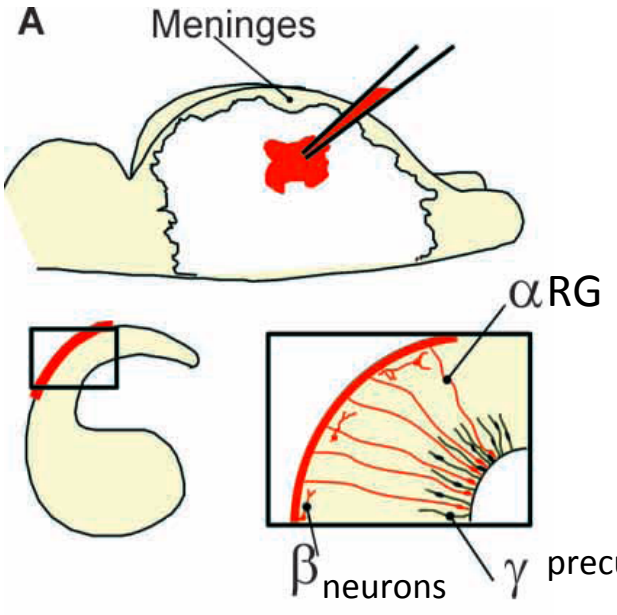
Examples of the progeny of hGFAP-GFP- and GLASTpositive precursor cells isolated by FACS after 5-7 div

cells were cultured on a rat cortex feeder layer of the corresponding age and identified by the mouse-specific antibody M2M6

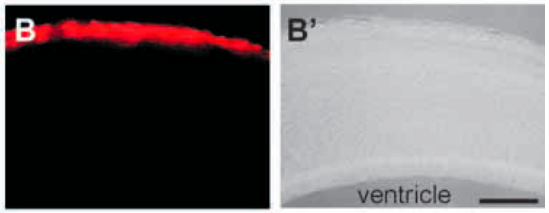


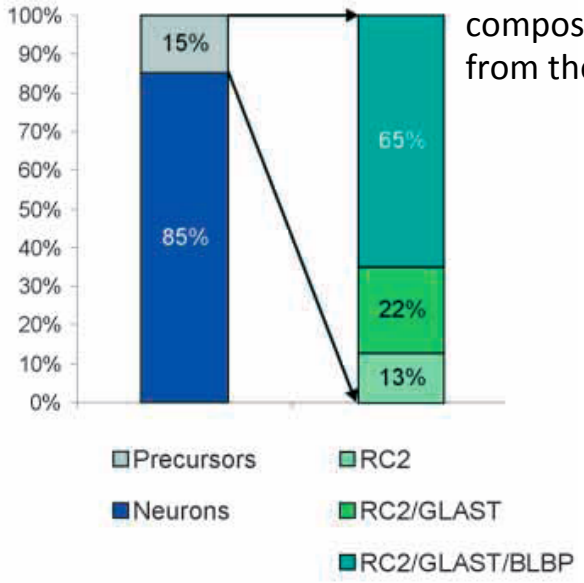
Cluster=clone

Morphological tracing of precursors with long radial processes:
Fluorescent tracing of cells from the pial surface



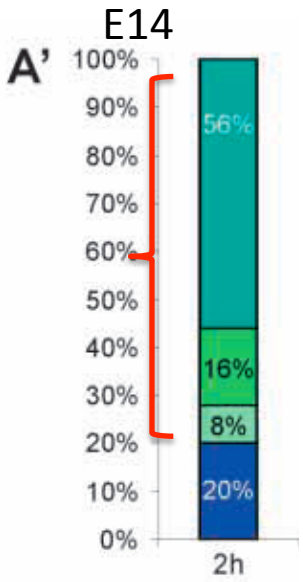
application of the fluorescent tracer DiI on the surface of the cortex (after meningeal removal) labels cells in contact with this surface, namely neurons and radial glial cells





composition of sorted cells labeled from the pial surface of E14 cortex

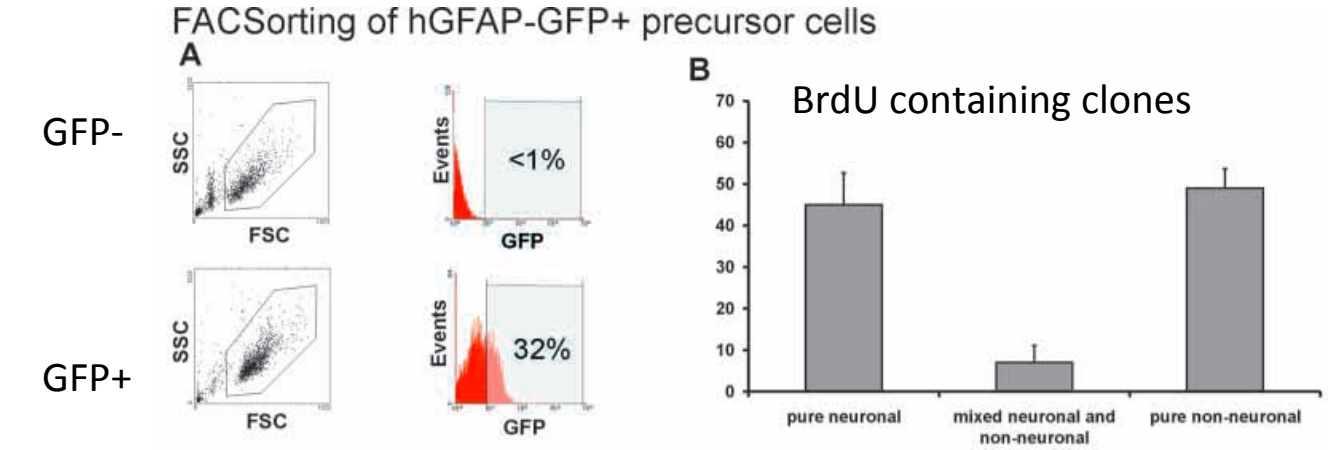
Note the similarity in the composition of precursors sorted by their morphology via pial tracing and/or their GFP-content driven by the hGFAP promoter

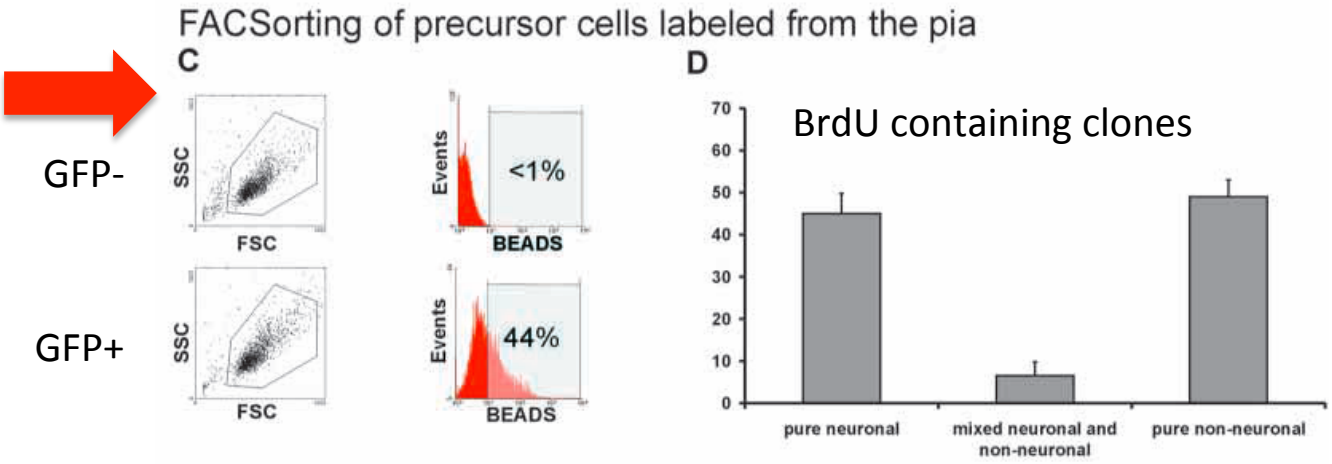


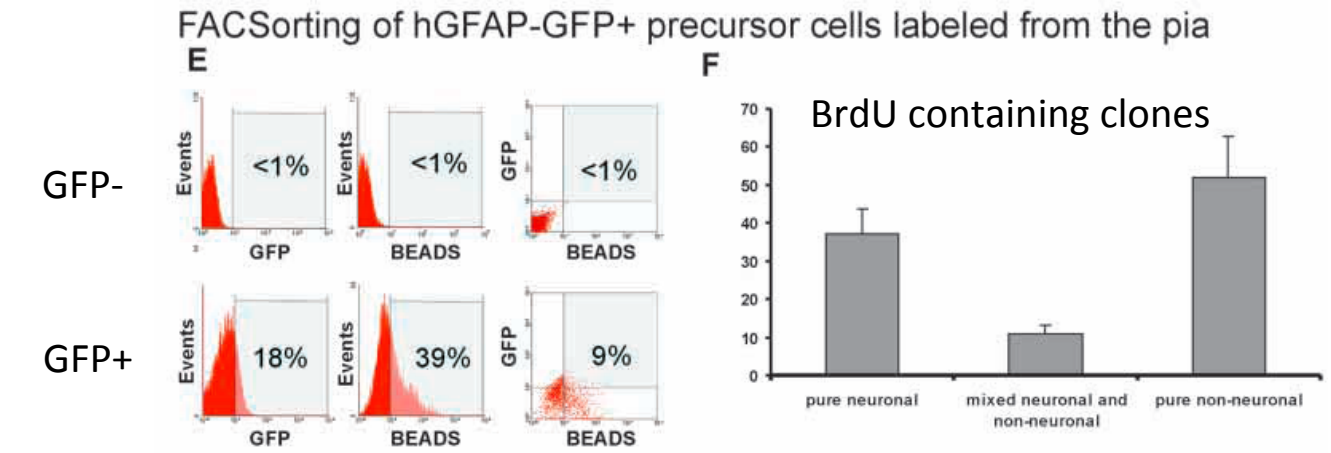
from E14 cortex

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

why???







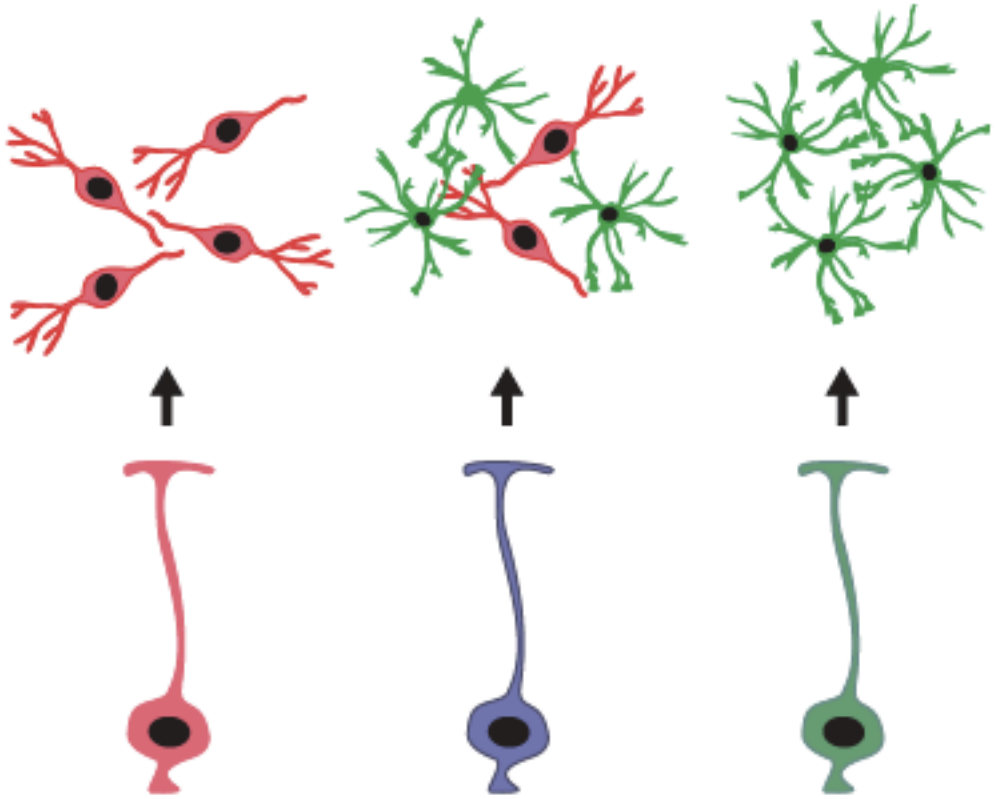


Fig. 2. Schematic summarizing the results of clonal analysis of single radial glial cells isolated by FACS as described in our *Development* paper in 2000 (Malatesta et al., 2000). Clones generated by a single cell were either composed of only neurons (red), of only glial cells (green) or of both glia 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).

Malatesta & Gotz, 2013

Neurons derived from radial glial cells establish radial units in neocortex

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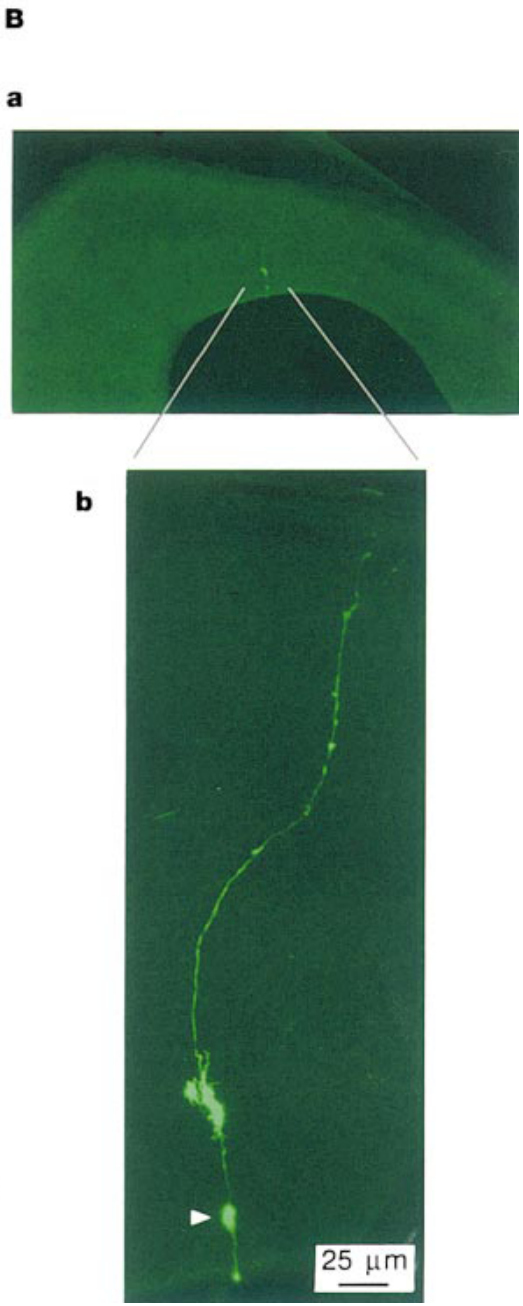
NATURE | VOL 409 | 8 FEBRUARY 2001 | www.nature.com



This study used low titres 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

A

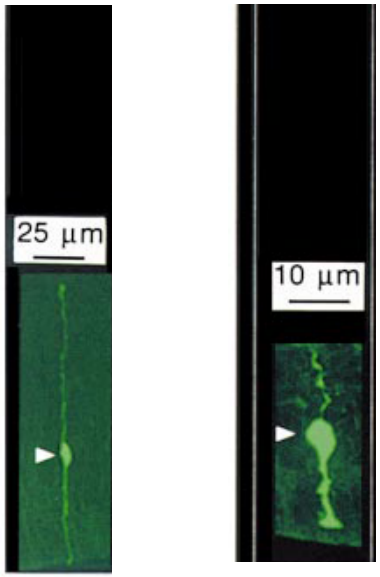
Morphological characteristics of radial glial cells 24h after infection:

1) a nucleus in the VZ;

2) a short process extending to the ventricular surface with a large end-foot;

3) a fine radial process extending to the pial surface;

4) frequent contact with blood Vessels



24 h

The GFP-expressing retrovirus randomly integrates into one daughter cell after infection.

when all GFP-labelled cells in the sensorimotor cortex of a single embryo were examined at **24 h** after infection, 49.8% (485/973) were **radial glia-like cells** with cell bodies located in the VZ

At E15-16, the predominat type of cell division (asymmetric) generate one neuron per cell cycle.

one division after infection, 50% of labelled cells should be progenitors and 50% should be postmitotic neurons.

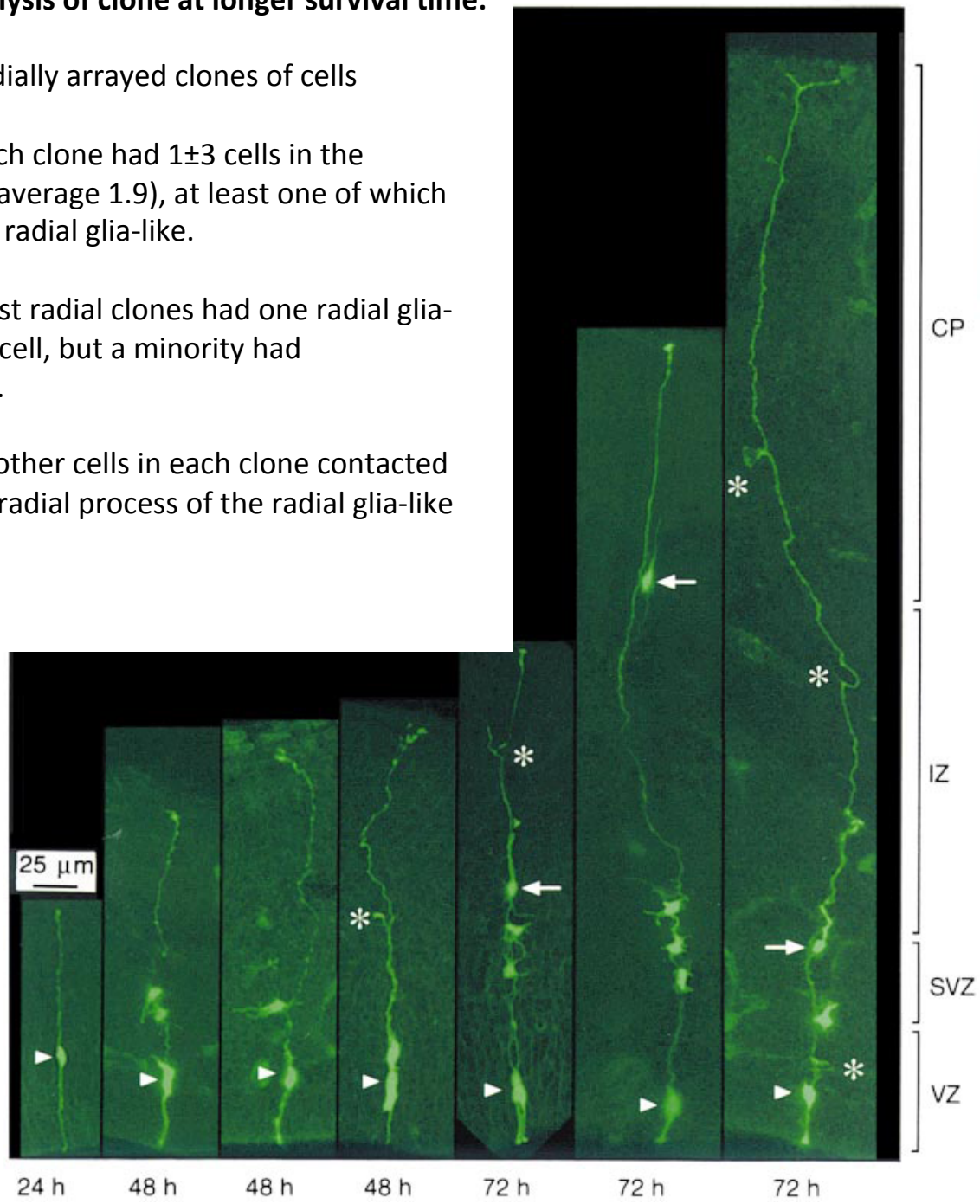
Analysis of clone at longer survival time:

- radially arrayed clones of cells

- each clone had 1±3 cells in the VZ (average 1.9), at least one of which was radial glia-like.

-most radial clones had one radial glia-like cell, but a minority had two.

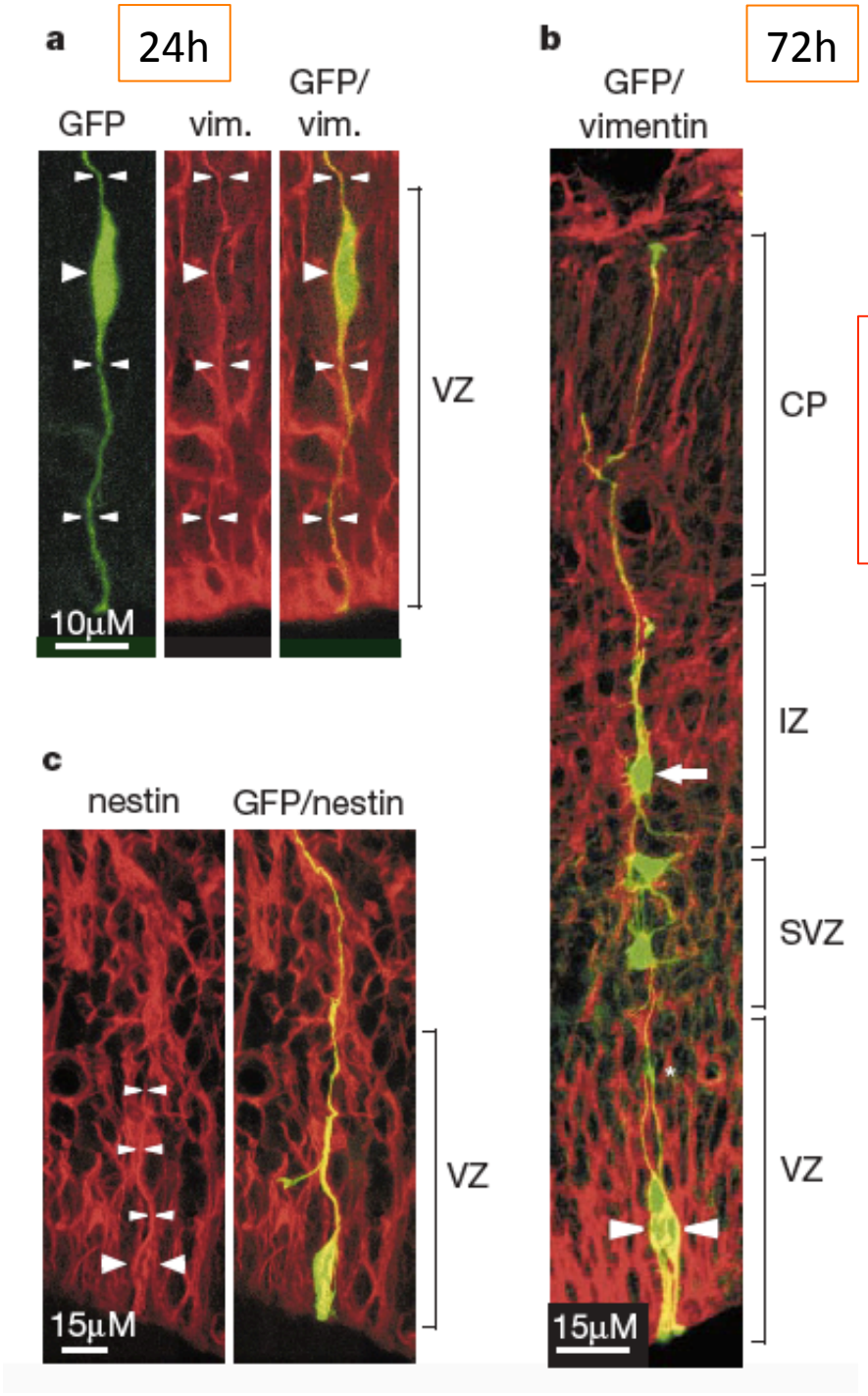
-all other cells in each clone contacted the radial process of the radial glia-like cell.



White arrow show migrating neurons (in the intermediate zone and cortical plate), with a **leading** and **trailing process** aligned along the GFP-labelled radial fibre

individual clones contain cells of mixed identity, including radial glial cells as well as immature neurons,

→ migrating neurons use clonally related radial glia as guides during migration.

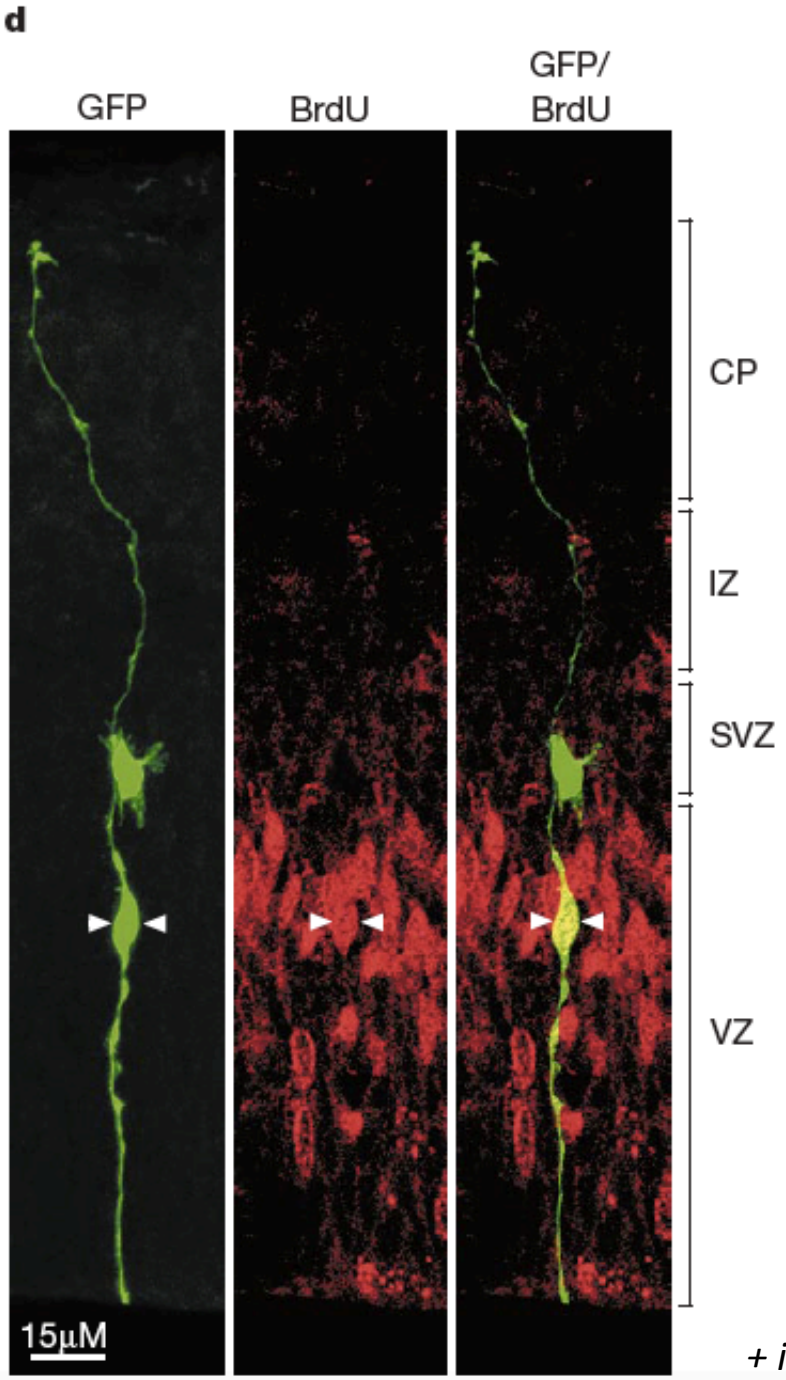


Immunolableling on fixed tissue

The incorporation of retrovirus by radial glial cells, as well as their expression of nestin, supports the concept that the radial glial cells in labelled clones are mitotically active

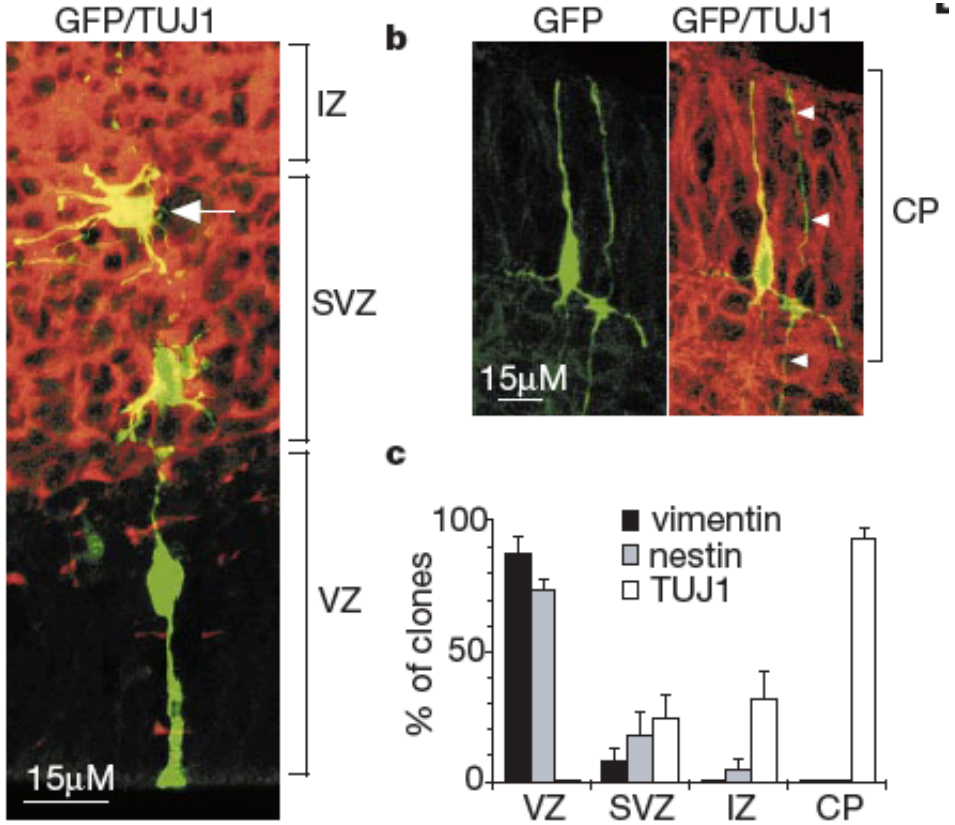
Vimentin is an intermediate filament protein expressed by radial glial cells

Nestin is expressed by proliferative neuroepithelial cells, including neuronal precursors



Radial glial cells are mitotically active during the period of neurogenesis and undergo interkinetic nuclear migration

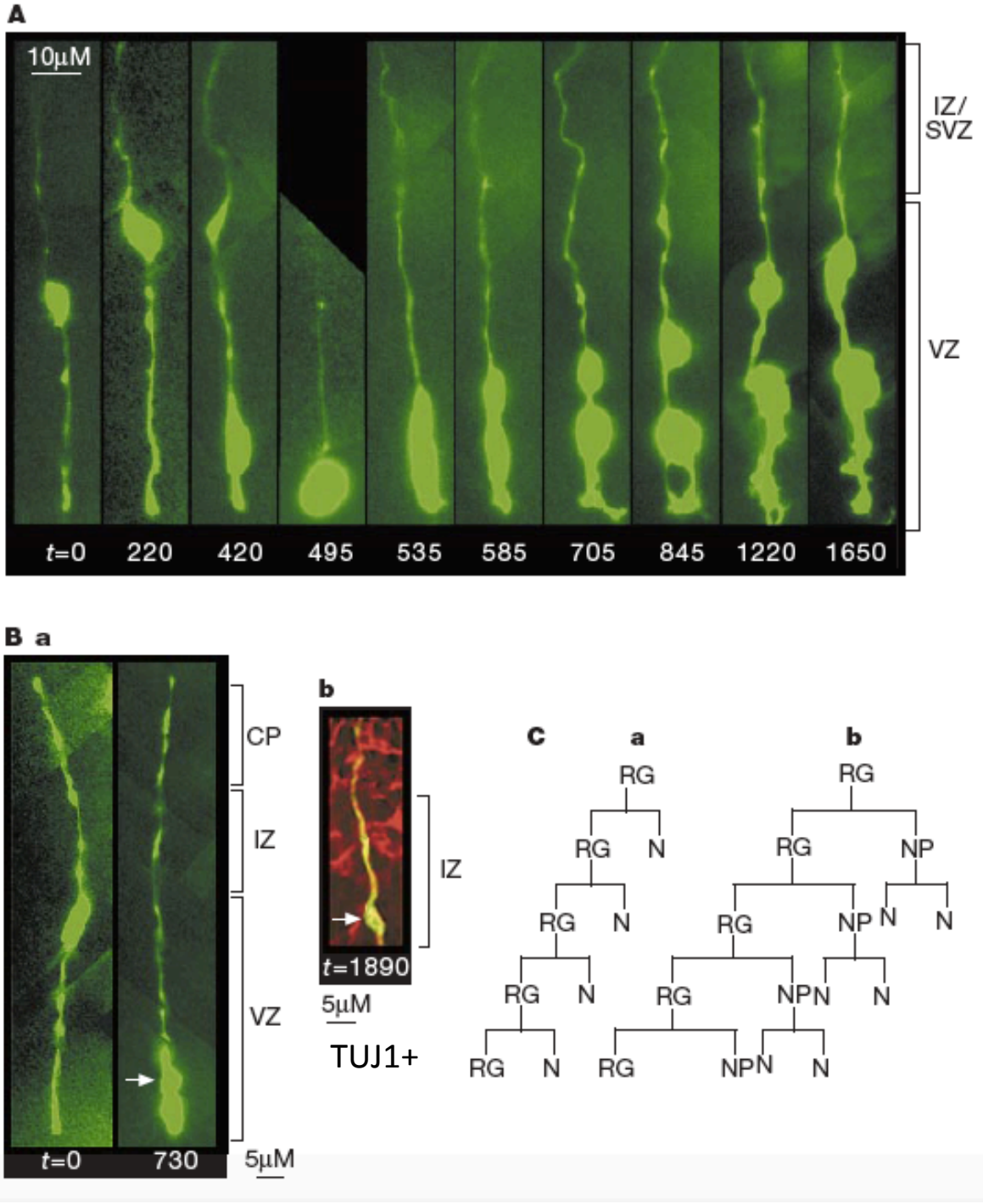
Radial clones contain radial glia and immature neurons (TUJ1+)



+ in living brain slices, electrophysiological recordings

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.



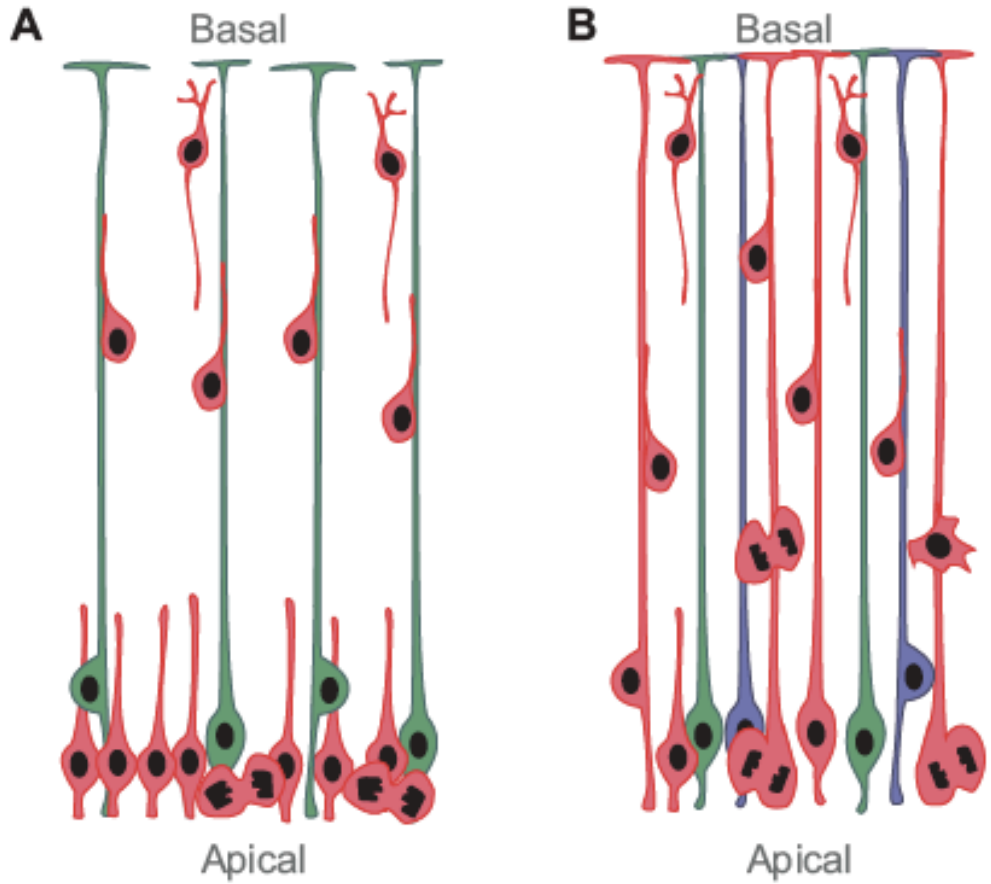


Fig. 1. Comparison between the 'old' and 'new' models of corticogenesis. (**A**) In the traditional view, radial glia (cells with long processes that span the whole apicobasal axis of the neuroepithelium; shown in green), were viewed as cables for guiding migrating neurons (red), and were thought to transform into glial cells at later stages of development. (**B**) The discovery that radial glia were proliferative and gave rise to neurons led to a new concept whereby radial glial cells are the majority of stem and progenitor cells in the ventricular zone, comprising specified neuronal (red) and glial (green) progenitors as well as bi-/multi-potent stem cells (purple).

2004

nature neuroscience

Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases

Stephen C Noctor[1], Verónica Martínez-Cerdeño[1], Lidija Ivic[1] & Arnold R Kriegstein[1–3]

Precise patterns of cell division and migration are crucial to transform the neuroepithelium of the embryonic forebrain into the adult cerebral cortex. Using time-lapse imaging of clonal cells in rat cortex over several generations, we show here that neurons are generated in two proliferative zones by distinct patterns of division. Neurons arise directly from radial glial cells in the ventricular zone (VZ) and indirectly from intermediate progenitor cells in the subventricular zone (SVZ). Furthermore, newborn neurons do not migrate directly to the cortex; instead, most exhibit four distinct phases of migration, including a phase of retrograde movement toward the ventricle before migration to the cortical plate. These findings provide a comprehensive and new view of the dynamics of cortical neurogenesis and migration.

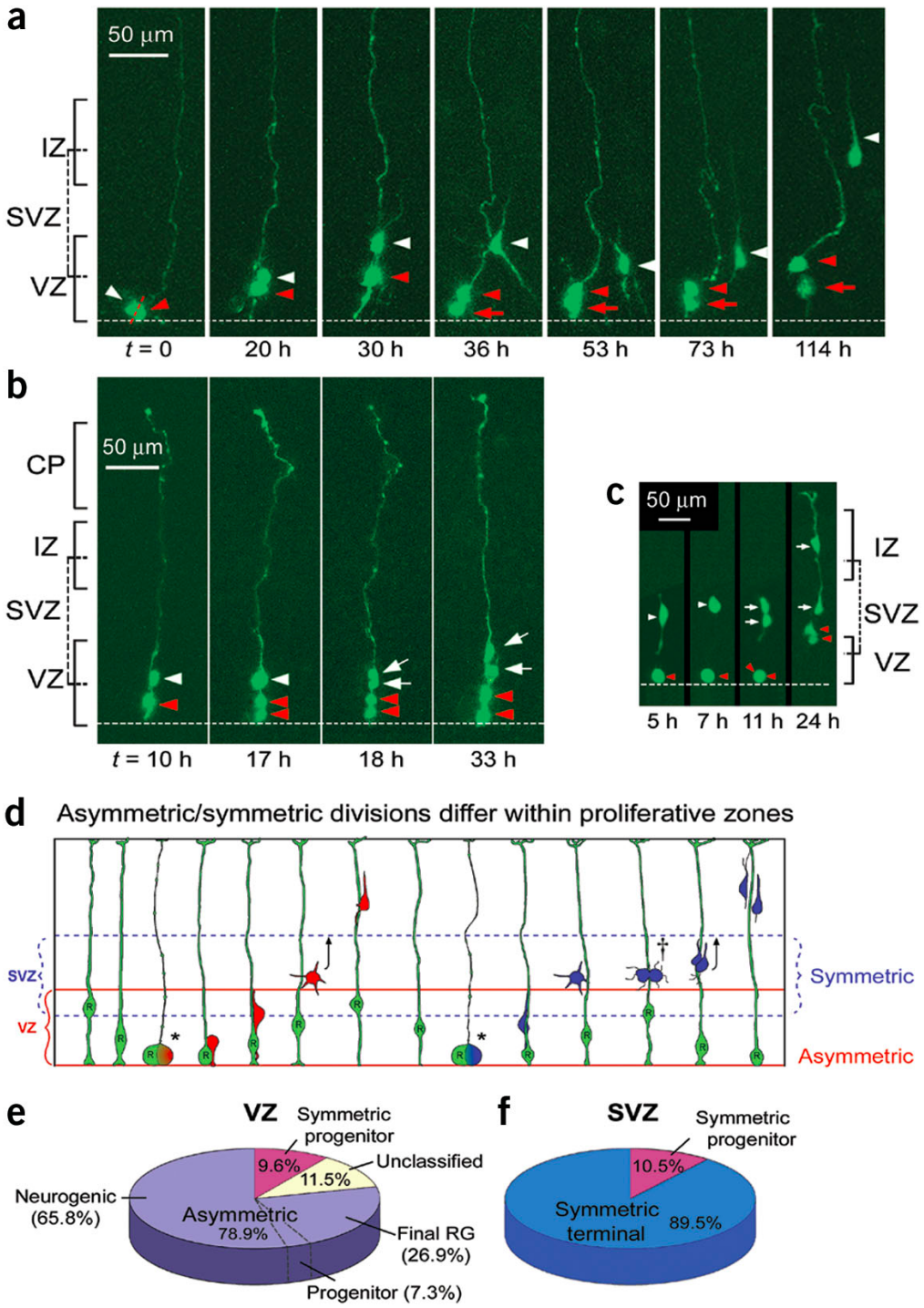
Retroviral labeling + time-lapse imaging

(precursor cell division at E17-E19 in rat embryos)

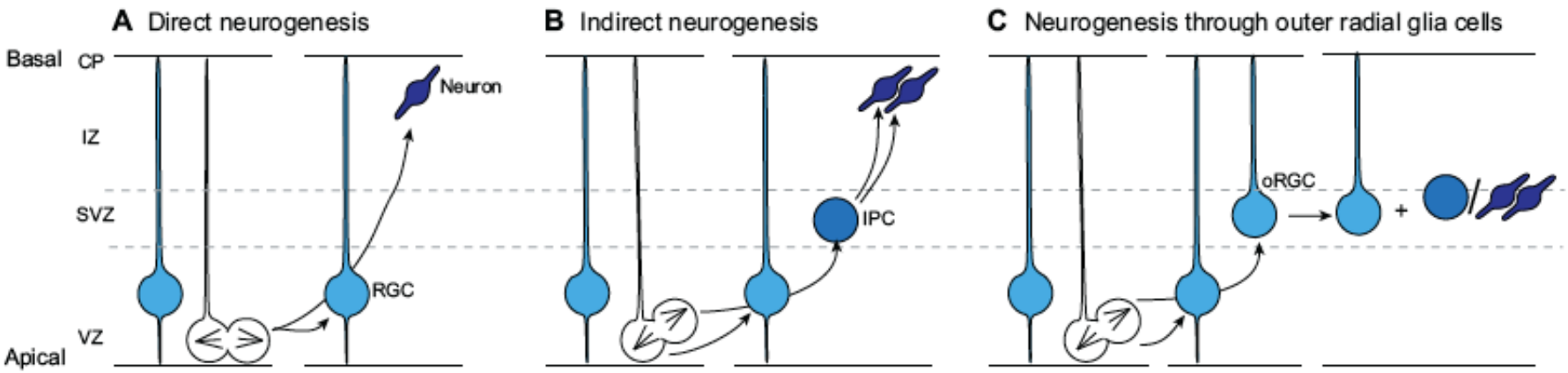
Radial glial cells divide asymmetrically in the ventricular zone (VZ),

Intermediate progenitor cells divide symmetrically in the subventricular zone (SVZ).

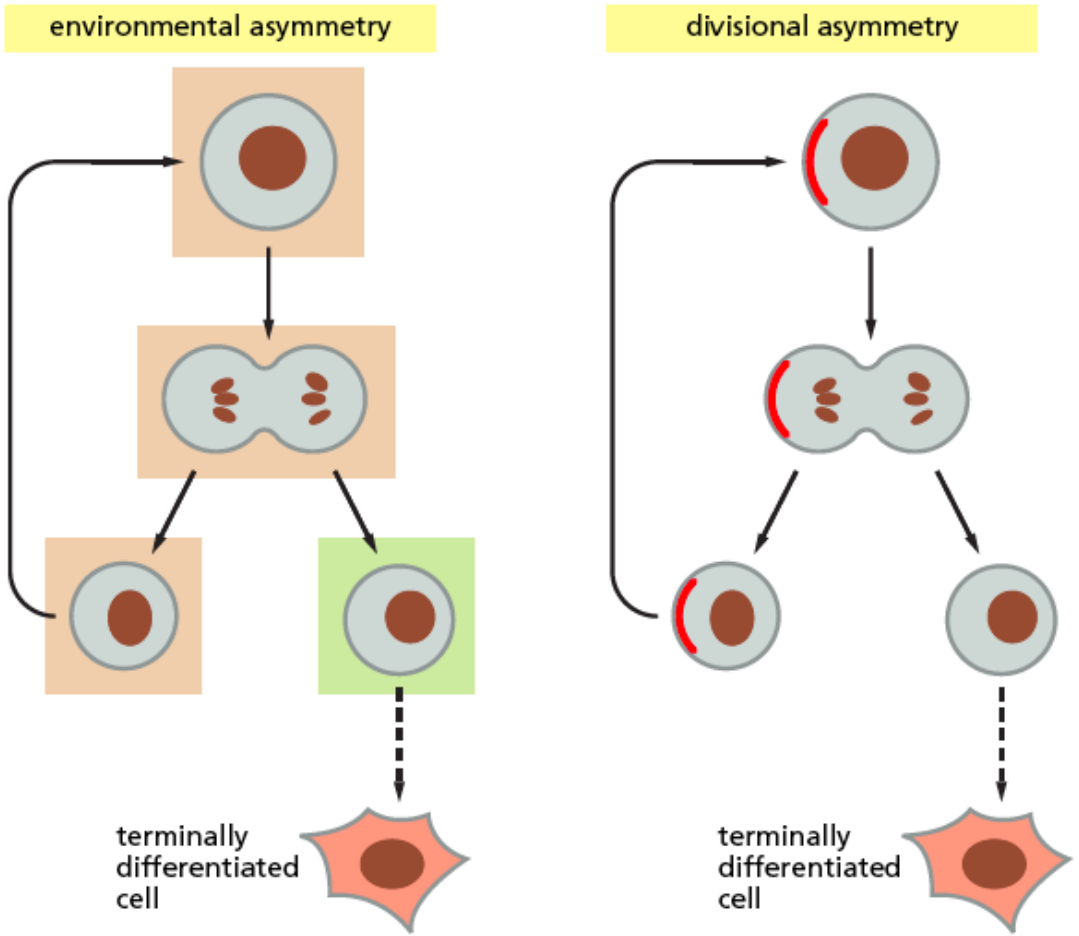
watch the VIDEO



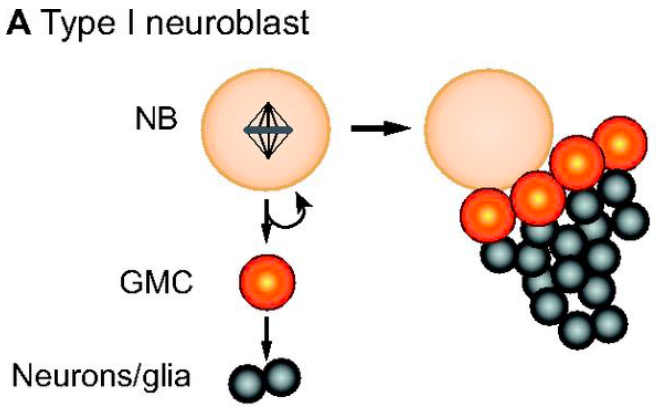
Development of the mouse neocortex



Symmetric vs asymmetric …stochastic or….



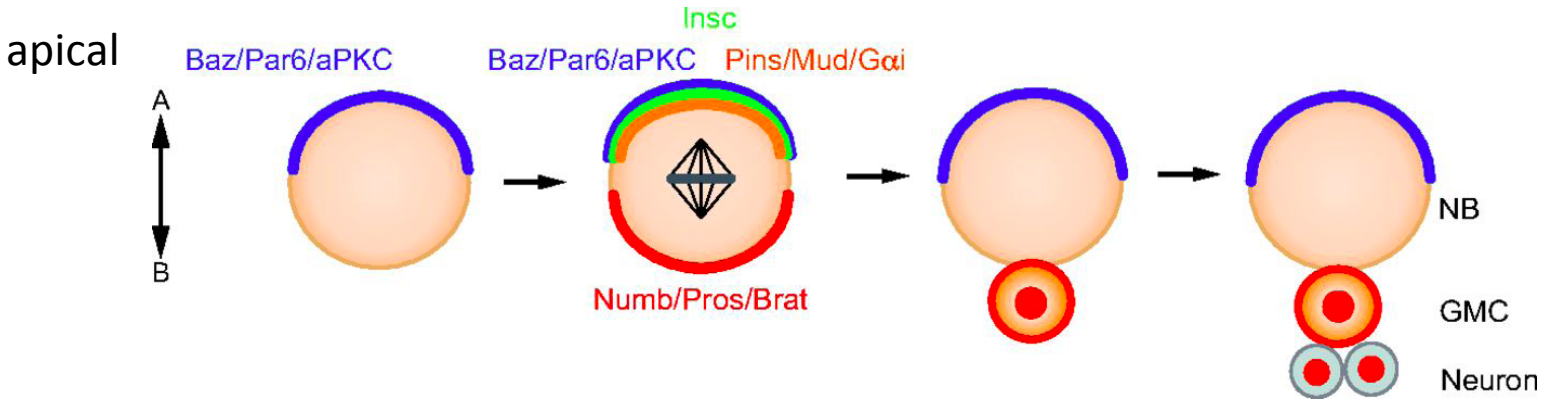
Neurogenesis in Drosophila



Steps in asymmetric neuroblast division:

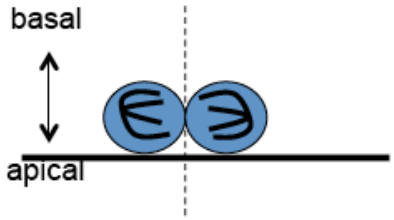
1. Establishment of polarity axis during interphase
2. Asymmetric localization of cell fate determinants in the neuroblasts
3. Appropriate spindle orientation during the onset of mitosis

4. Differential segregation of cell fate determinants between the two daughter cells.

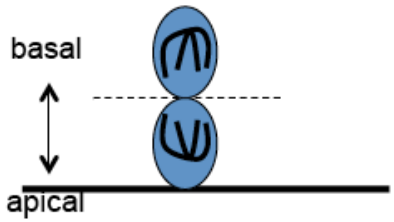


Asymmetric cell division is controlled intrinsically in NBs

Classical Drosophila concept



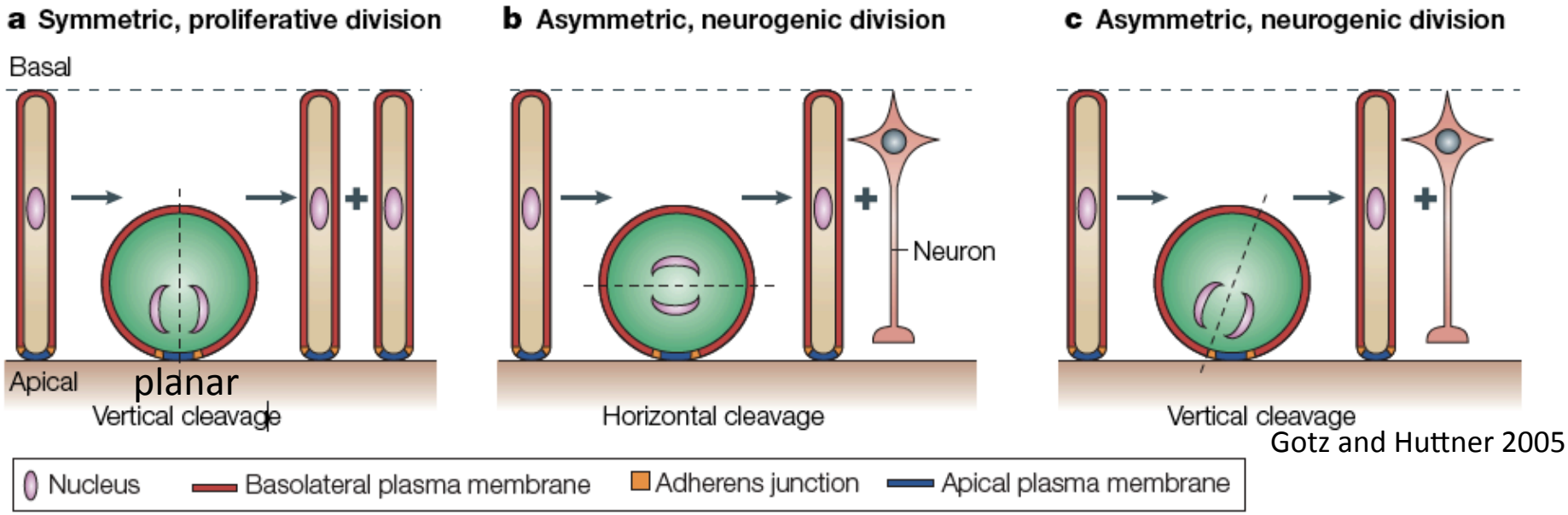
Symmetric (planar) division:
Vertical cleavage plan
Two identical cells



Asymmetric (apical/basal) division:
Horizontal cleavage plan
Two different cell types

Symmetrical-asymmetrical division of NPs and cleavage plane

Homologues of the molecules involved in spindle orientation in invertebrate models have been identified in vertebrates and they are all expressed in neuroepithelial and radial glial cells



Gotz and Huttner 2005

Bisect or **bypass** model

Unlike in *Drosophila*, in mammals, RG divisions can be asymmetric even when the mitotic spindle is almost parallel to the apical surface and the two daughter cells are of equal size

asymmetric segregation of apically localized cell fate determinants could still occur without apico-basal division, thanks to the small size of the apical domain

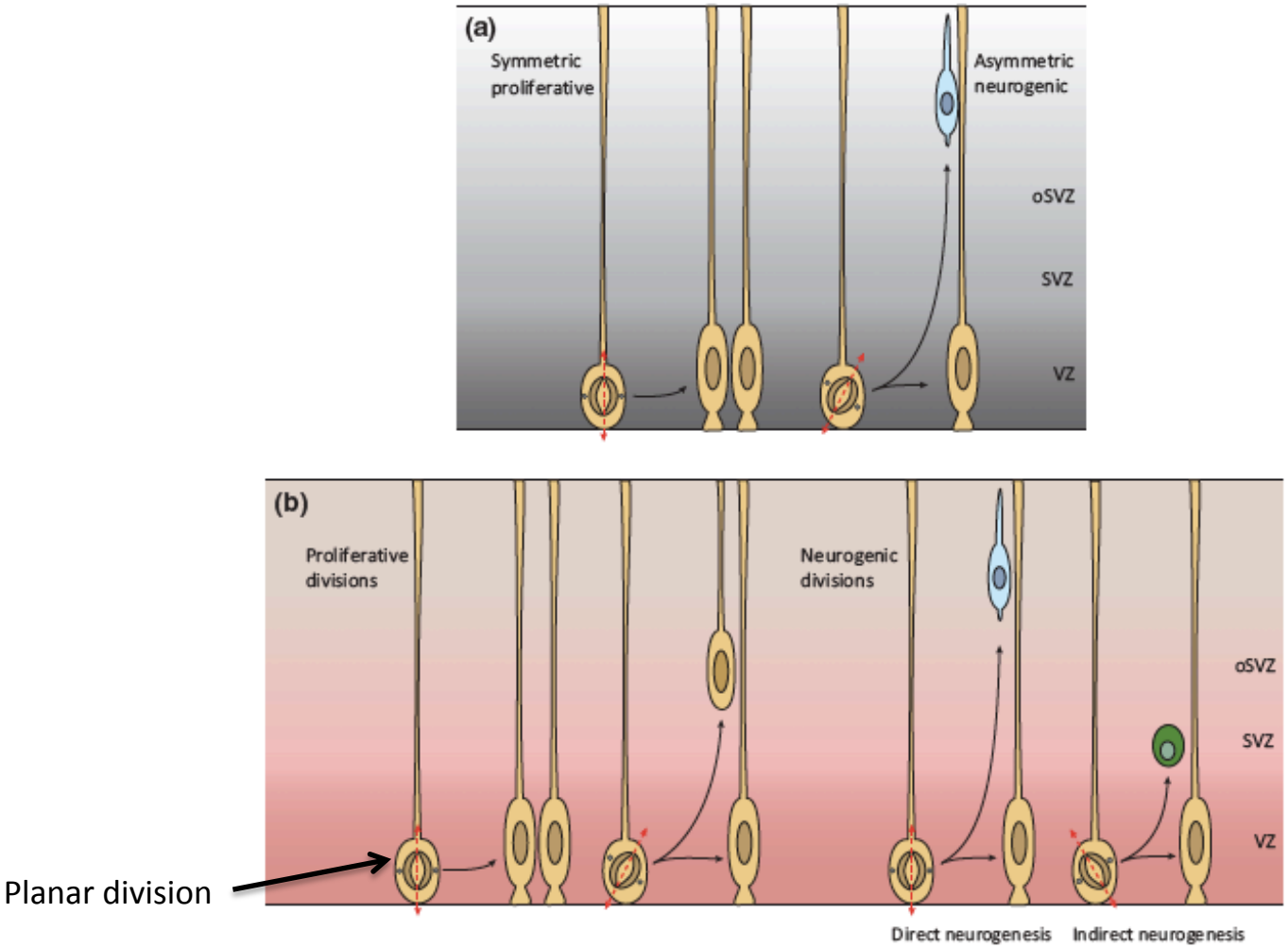
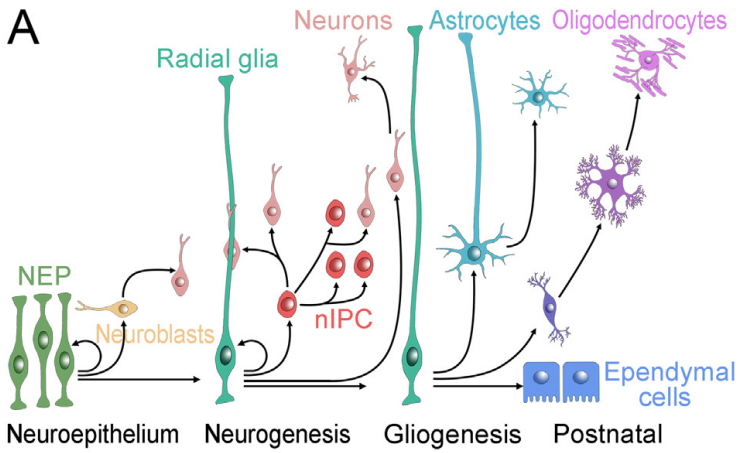


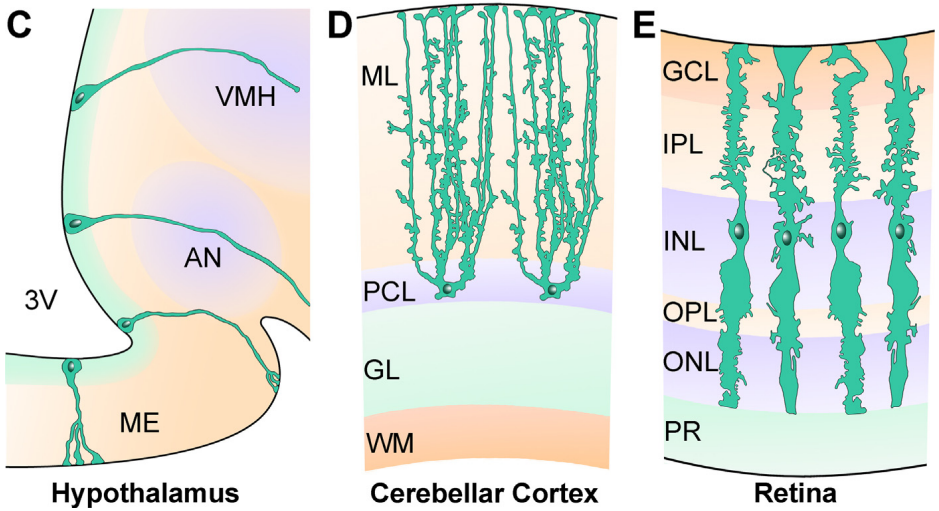
Fig. 3. Historical perspective and current view of the role of spindle orientation in cell fate acquisition. (a) An historical model proposed a causal relationship between spindle orientation and the choice between symmetric versus asymmetric divisions. Horizontal divisions would give rise to equally fated daughter cells (progenitor cells depicted here). Oblique or vertical divisions would give rise to daughter cells with different fate (here a progenitor and a committed cell). (b) New evidence has shown that both planar and oblique/vertical divisions can give rise to either symmetric proliferative or asymmetric neurogenic divisions. The orientation of the mitotic spindle could, however, influence the identity of daughter cells produced within proliferative or neurogenic lineages. In the case of proliferative divisions, planar divisions are thought to favor the production of two RG, whereas an oblique spindle may favor the generation of an oRG and a RG, as seen in LGN perturbation (Shitamukai *et al.* 2011). During the neurogenic phase, it is proposed that planar divisions correspond to direct neurogenesis and oblique divisions favor indirect neurogenesis through the generation of IP cells, as seen in Insc perturbation (Postiglione *et al.* 2011).

What is the fate of radial glial cells when neurogenesis comes to an end?

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



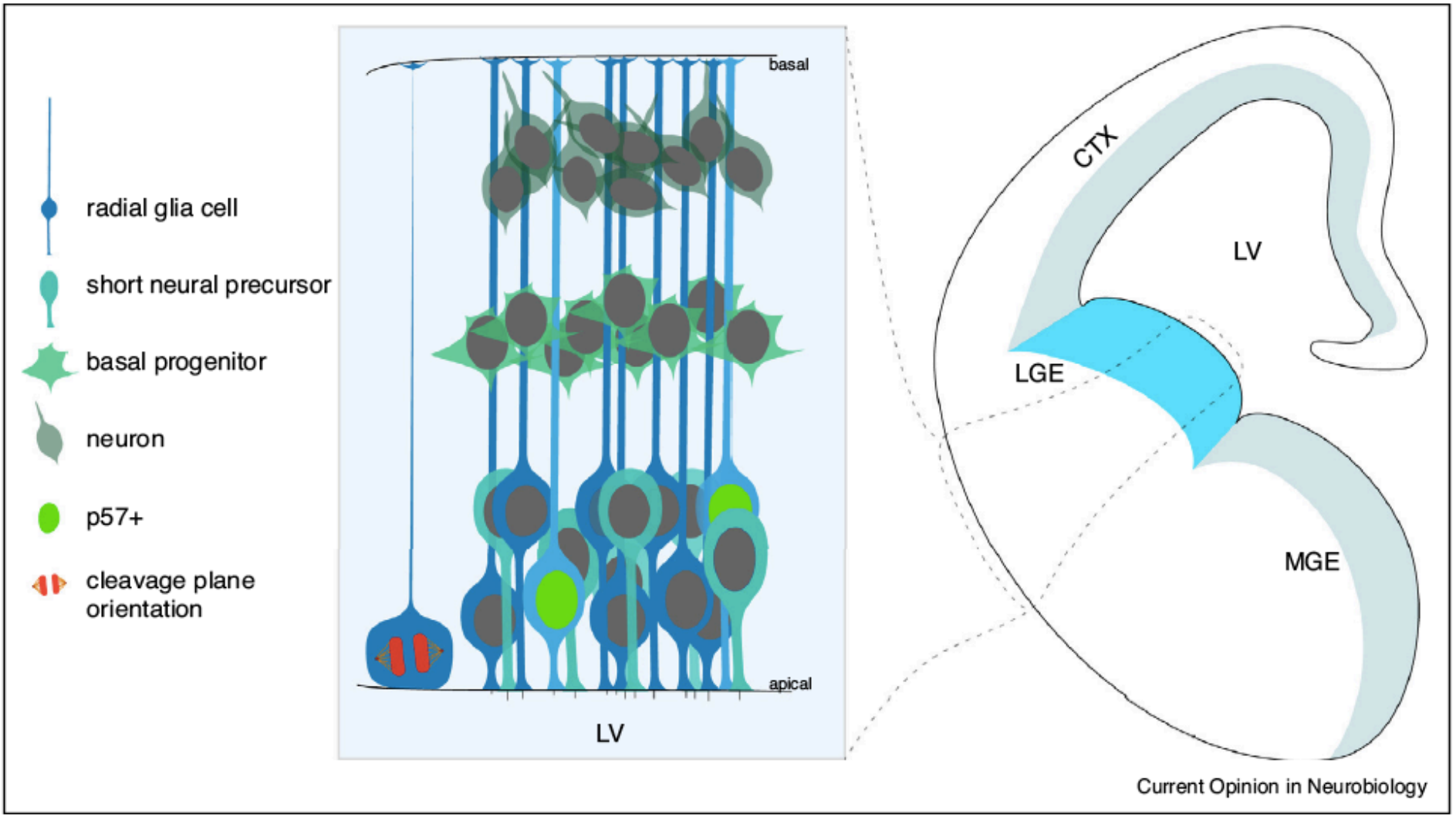
Adult



Tanycytes | Bergman glia | Müller cells

+ adult neurogenic astrocytes

In NON-mammalian vertebrates radial glial cells persist into the mature brain and continue to line the ventricle (act as progenitors for adult neurogenesis)



Embryonic origin of adult neural stem cells. Schematic representation of the origin of adult NSCs in the SEZ.

The adult NSCs residing in the SEZ are mainly derived from progenitor cells in the LGE. The number of adult NSCs is controlled by the **cleavage plane orientation** in RGCs during embryonic development and by the fraction of RGCs **upregulating p57** to drive them into quiescence. LV: lateral ventricle; CTX: cortex; MGE: medial ganglionic eminence; LGE: lateral ganglionic eminence.

Falk and Gotz 2017

Cell

Article

Embryonic Origin of Postnatal Neural Stem Cells

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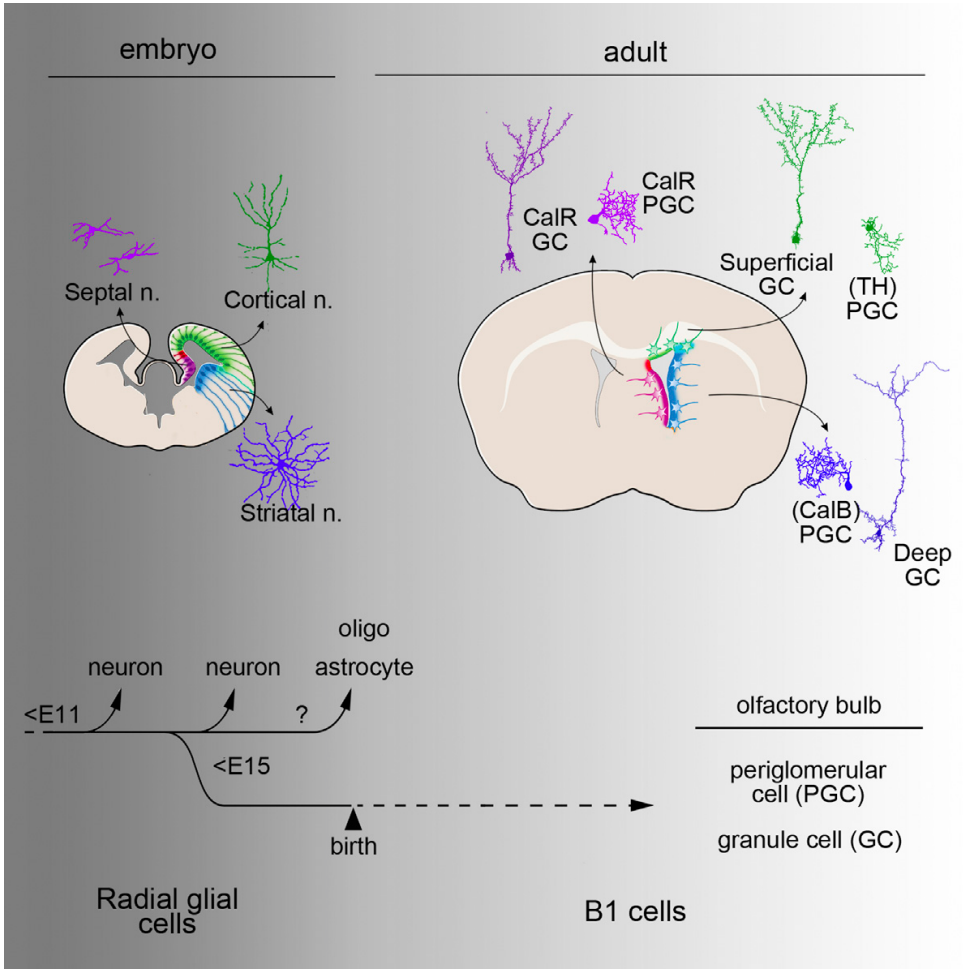


Here we show that the majority of mouse B1 cell precursors are produced between embryonic days (E) 13.5 and 15.5 and remain largely quiescent until they become reactivated postnatally.

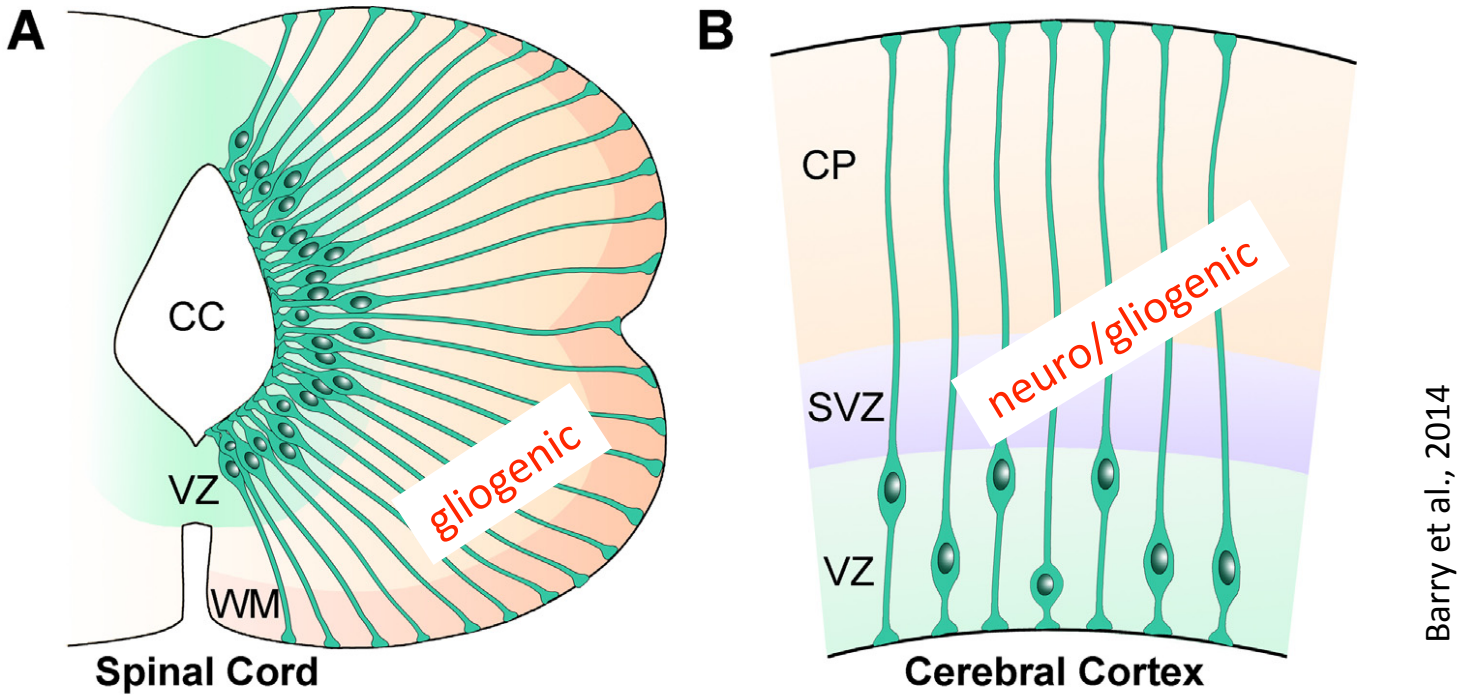
Using a retroviral library carrying over 100,000 genetic tags, we found that B1 cells share a common progenitor with embryonic cells of the cortex, striatum, and septum, but this lineage relationship is lost before E15.5.

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.

Radial glial cells

embyonic



Barry et al., 2014

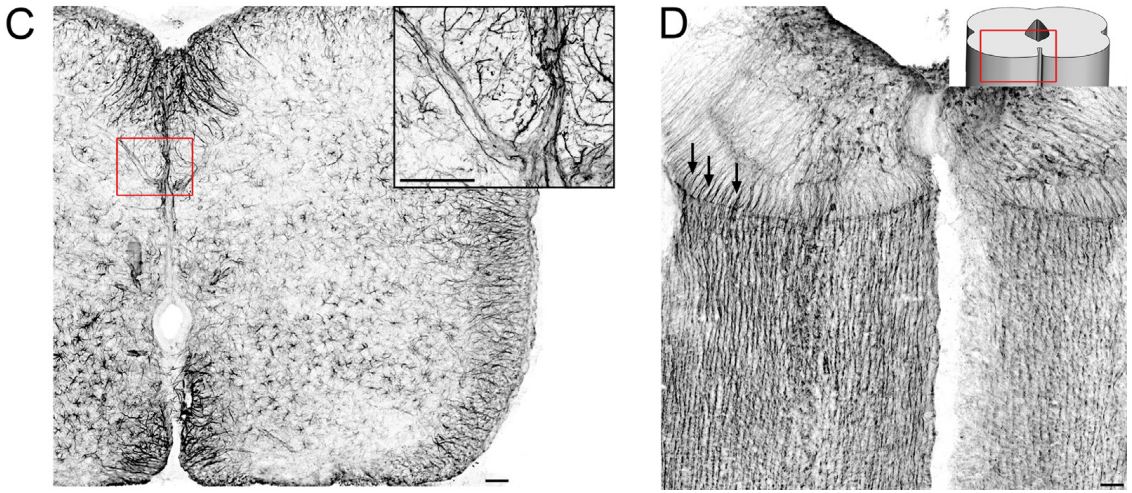
Highly heterogeneous (spatio – temporal)

In addition to the role as neural progenitors and migration…

facilitate the formation and compartmentalisation of the white matter:

→In the developing corpus callosum axons grow within a transient glial band composed of radial glia,
that disappears around the perinatal period

→In the spinal cord, finely organised radial glial processes create boundaries which separate nascent axon tracts in the emerging dorsal and lateral white matter



Spinal cord (RG-BLBP+)

-new evidence for a role in vascularization (cerebral cortex) (Ma et al., 2013)

Barry et al., 2014