ASTROGLIOGENESIS in the cerebellum

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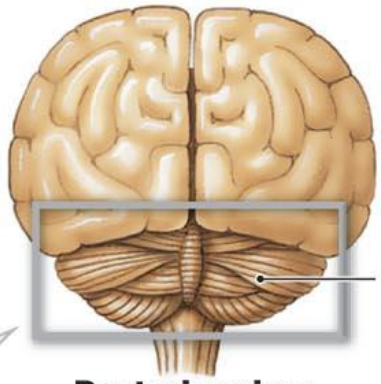
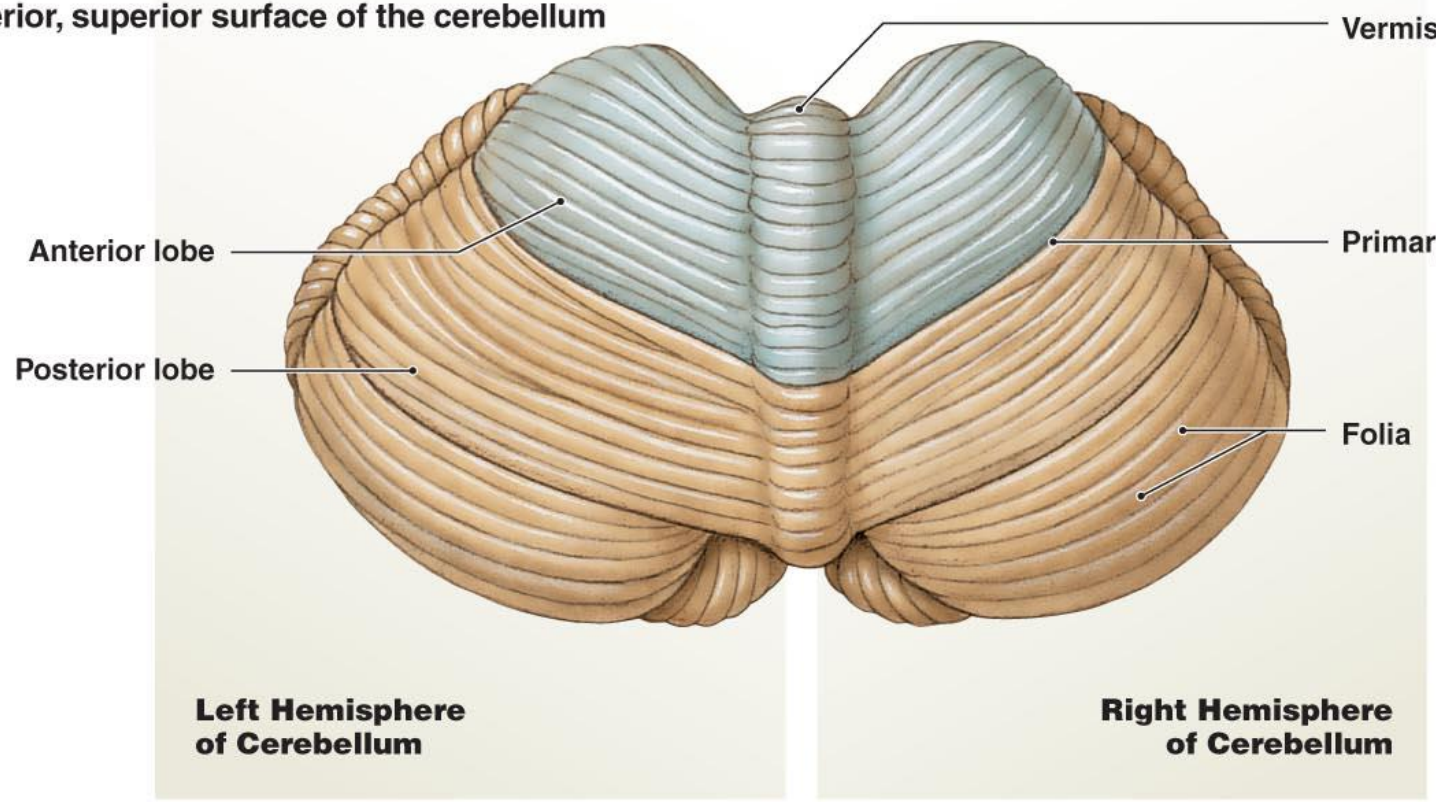
The cerebellum

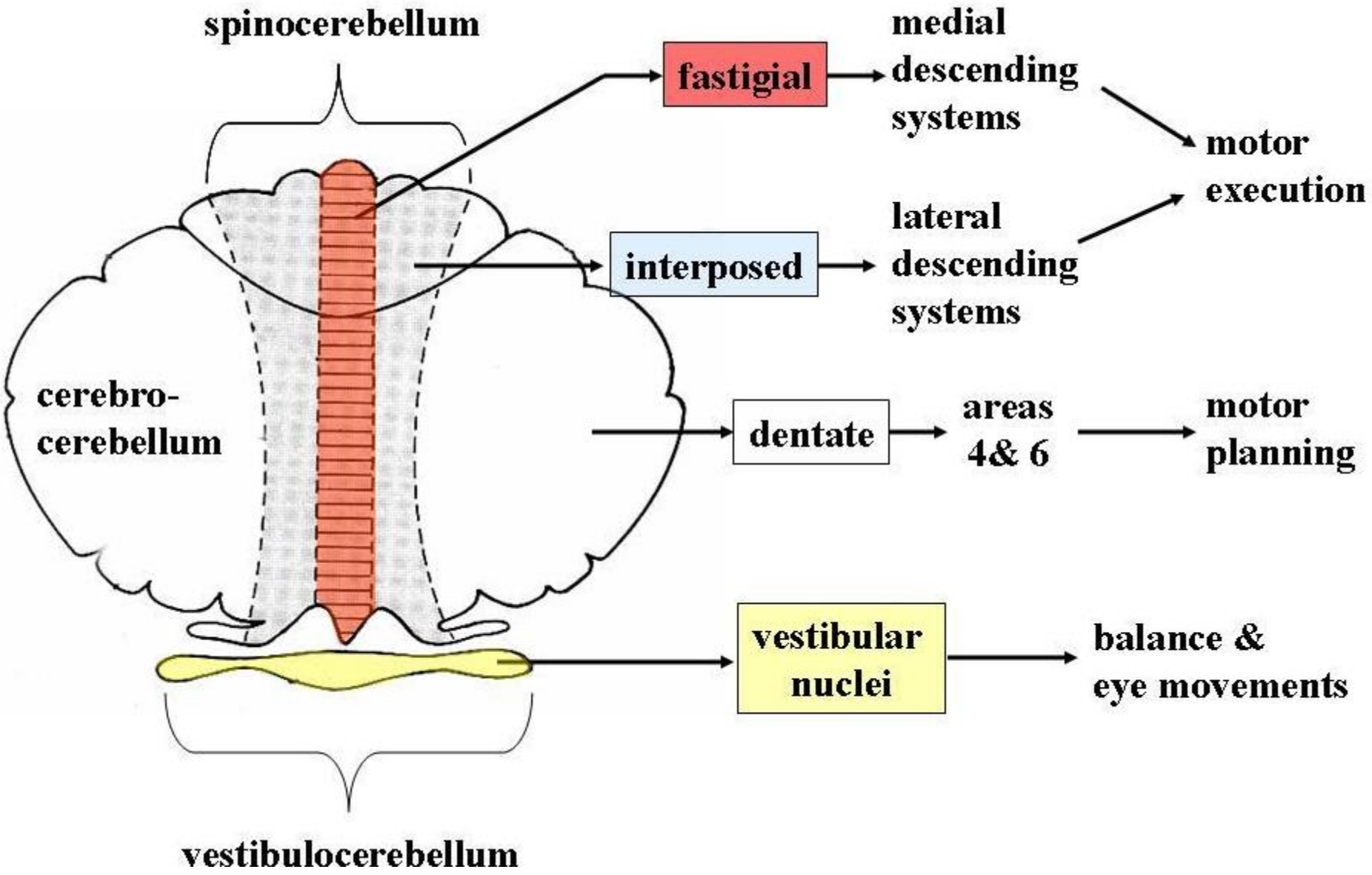


Structural features of the cerebellum

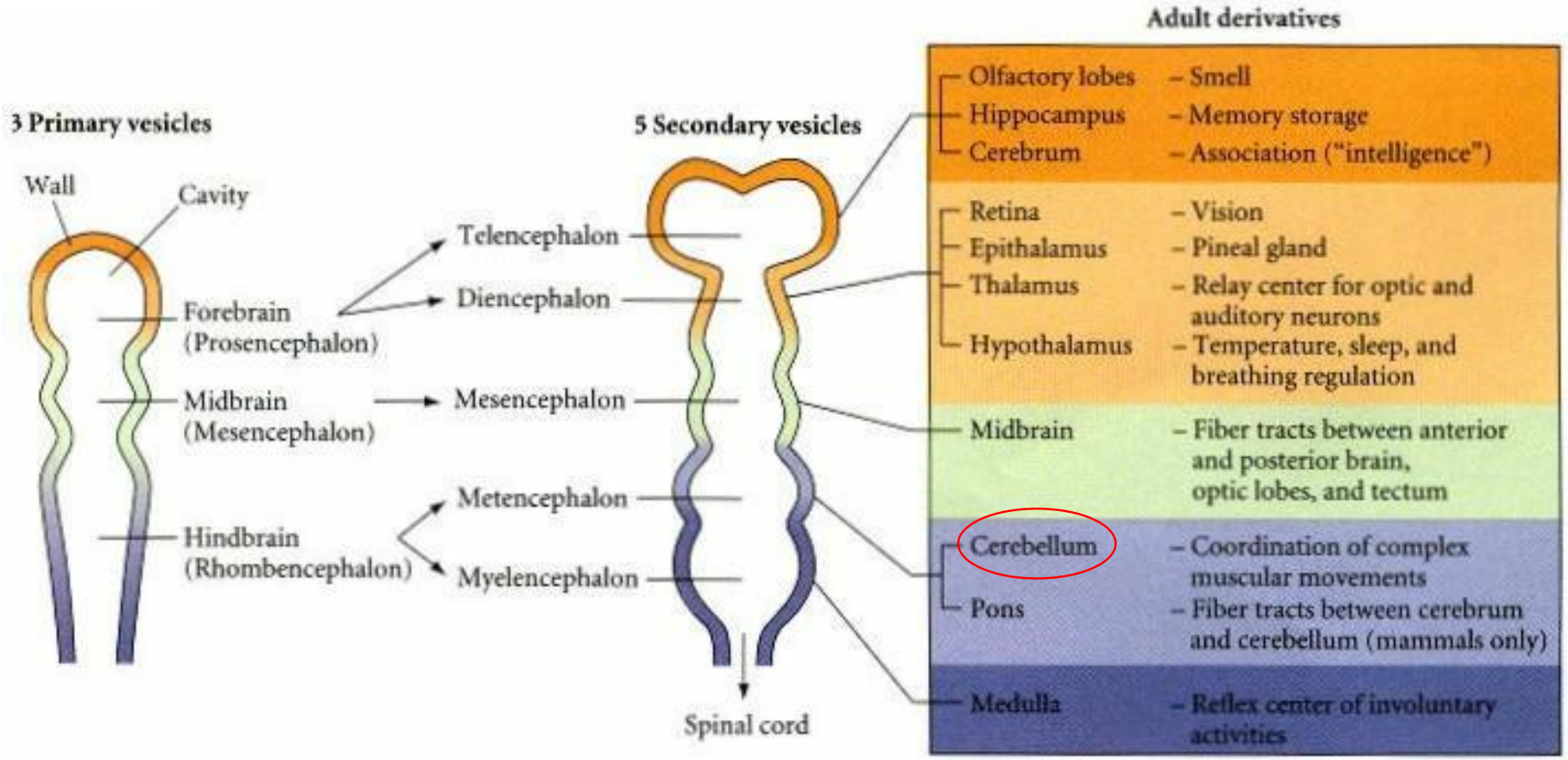
The posterior, superior surface of the cerebellum



Cerebellar functions

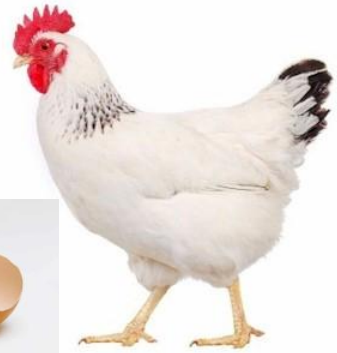


Neural tube patterning

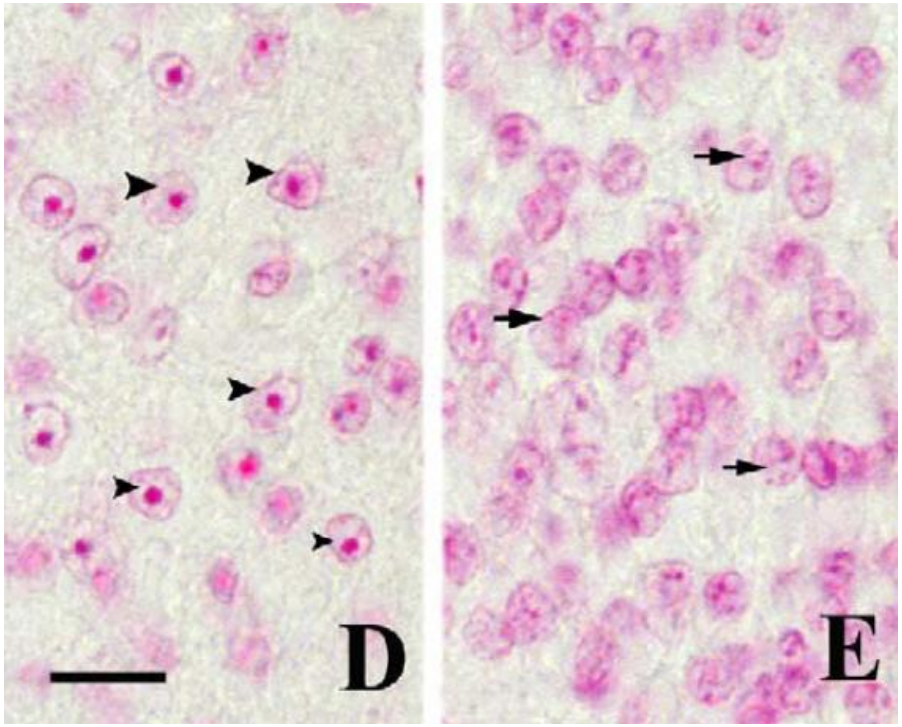


The origin of the cerebellar anlage

Quail-chick grafts



Quail
=
Condensed heterochromatin

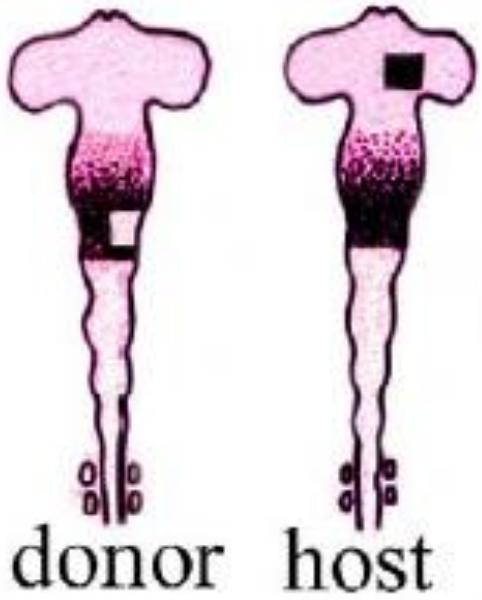


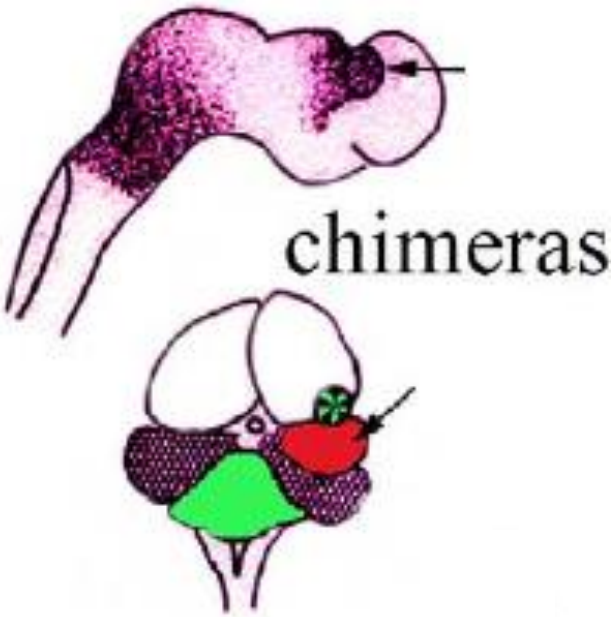
Chick
=
NOT Condensed heterochromatin

Martinez and Alvarado-Mallart, 1989;
Hallonet et al., 1990;
Hallonet and Alvarado-Mallart, 1997

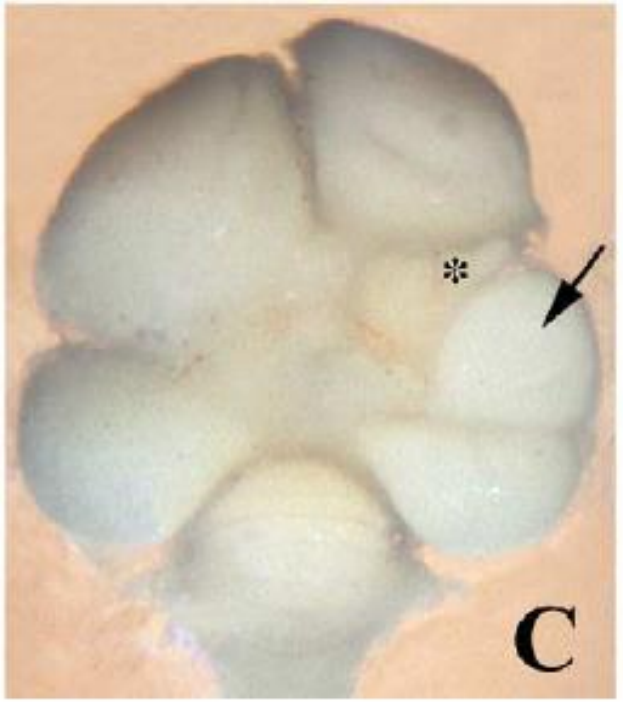
The origin of the cerebellar anlage

Quail-chick grafts





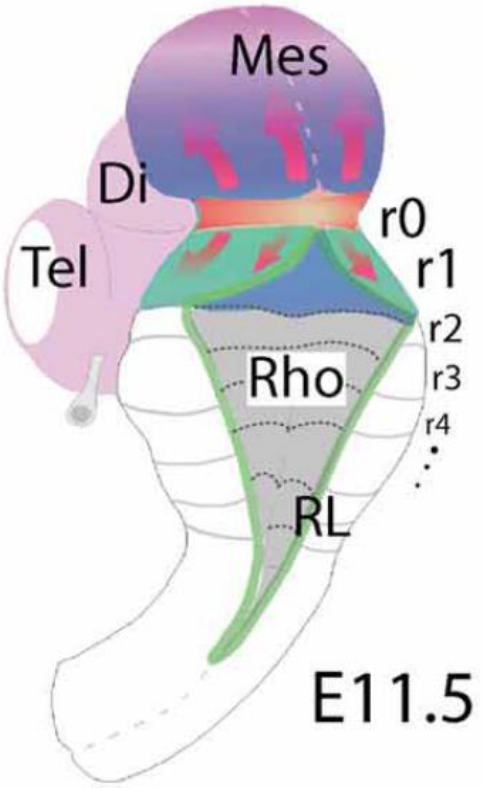
Homocronic and **hetero**topic grafts

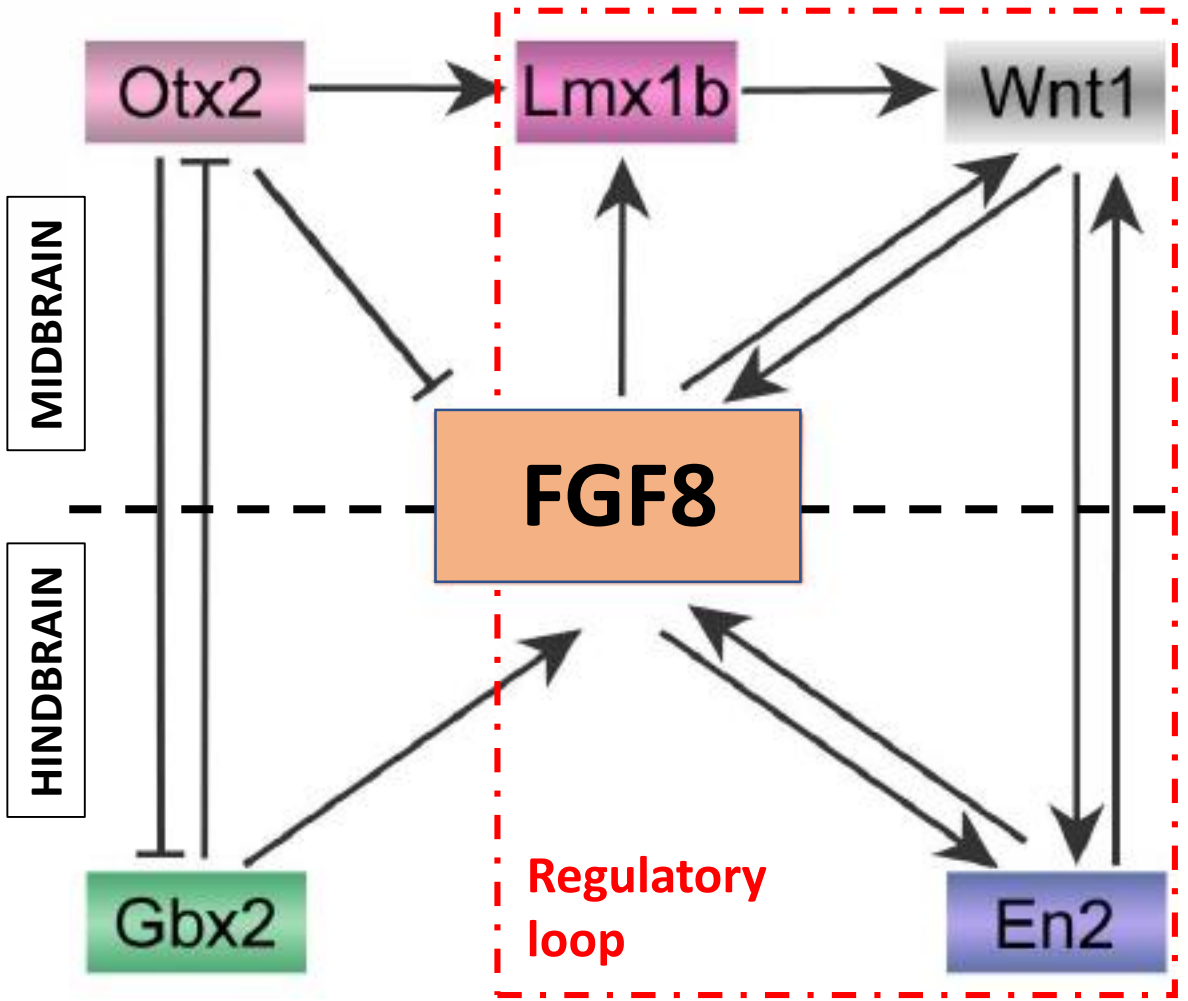


Both mesencephalon and metencephalon contribute to the developing cerebellum

Martinez and Alvarado-Mallart, 1989;
Hallonet et al., 1990;
Hallonet and Alvarado-Mallart, 1997

The «isthmic organizer» (IsO)

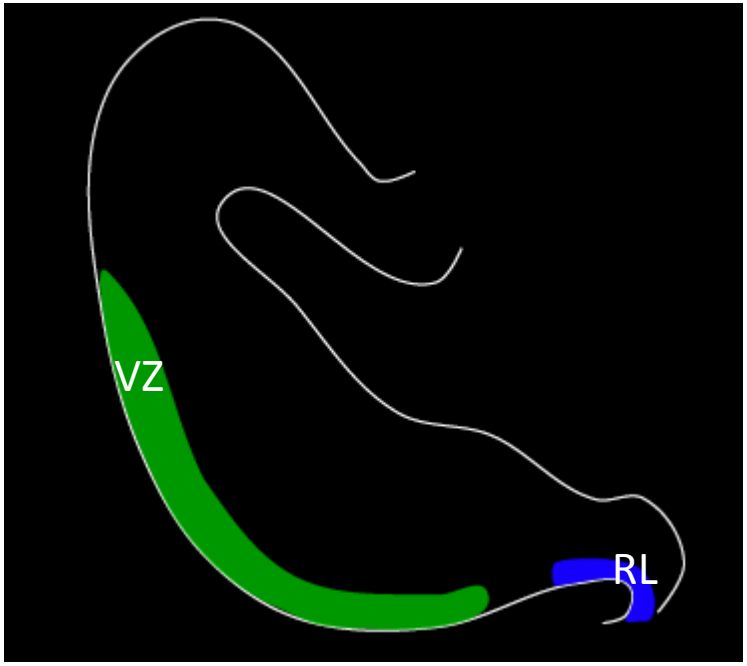




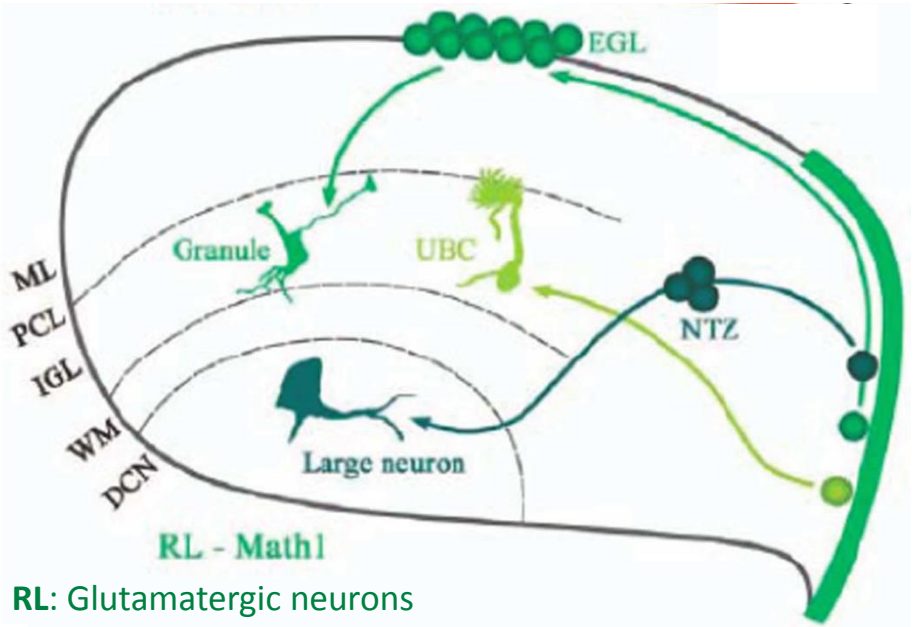
Timely and spatially regulated expression of specific molecular signals build the organizing activity of the IsO

Martinez et al. 2013;
Carletti and Rossi, 2008

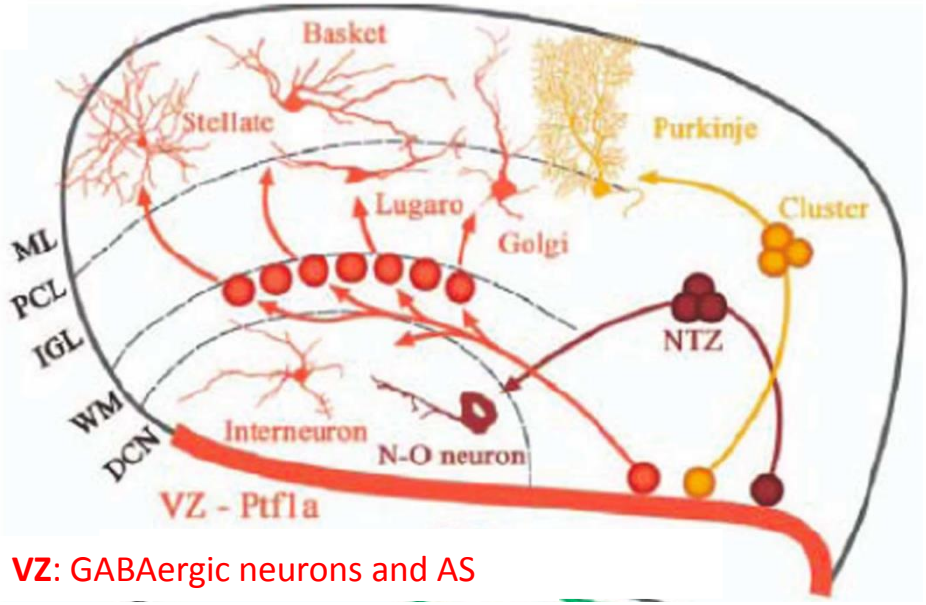
Cerebellar embryonic neuroepithelia



Embryonic development:
primary germinal sites

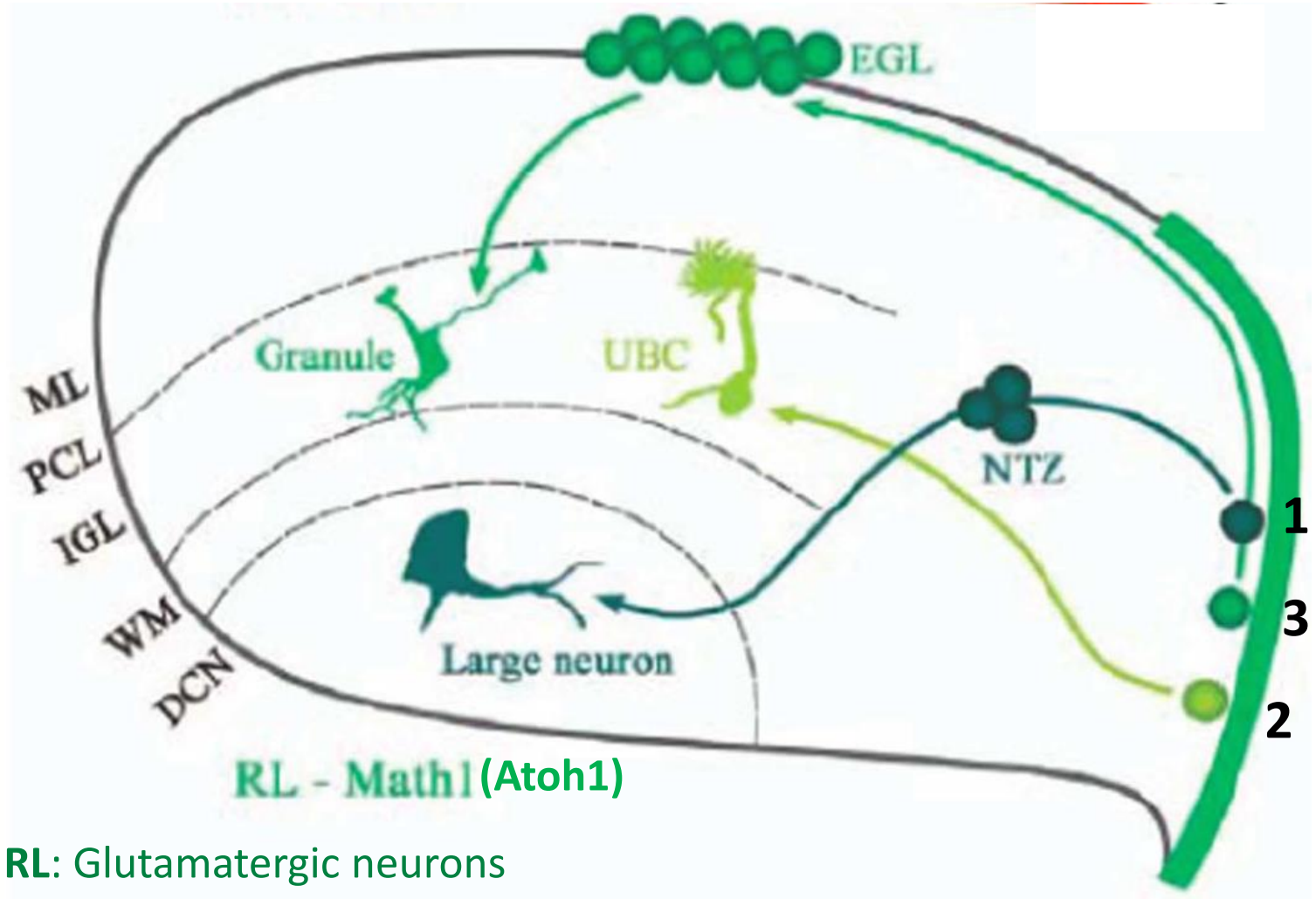


RL: Glutamatergic neurons



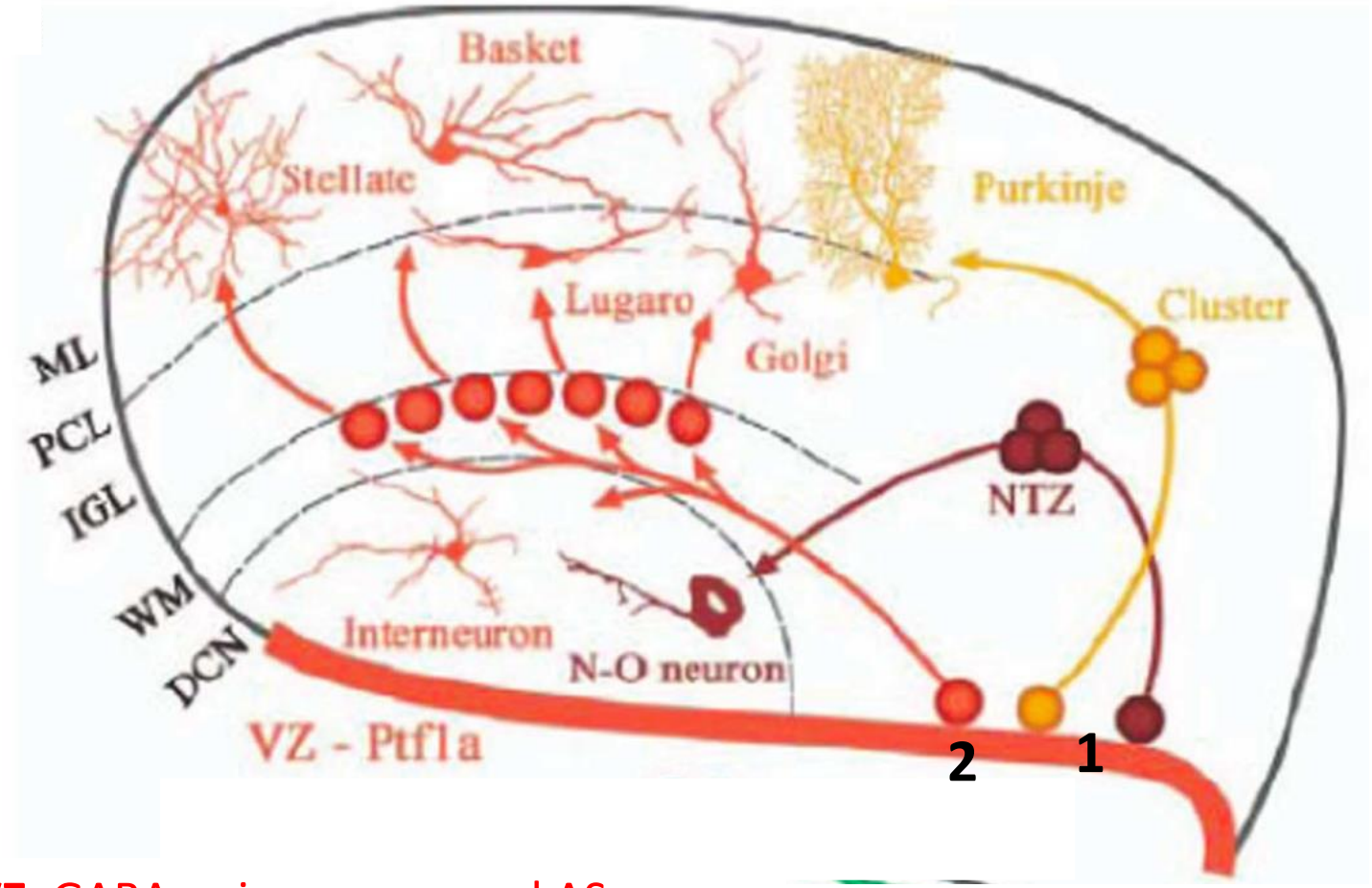
VZ: GABAergic neurons and AS

Cerebellar embryonic neuroepithelia: The Rhombic Lip (RL)



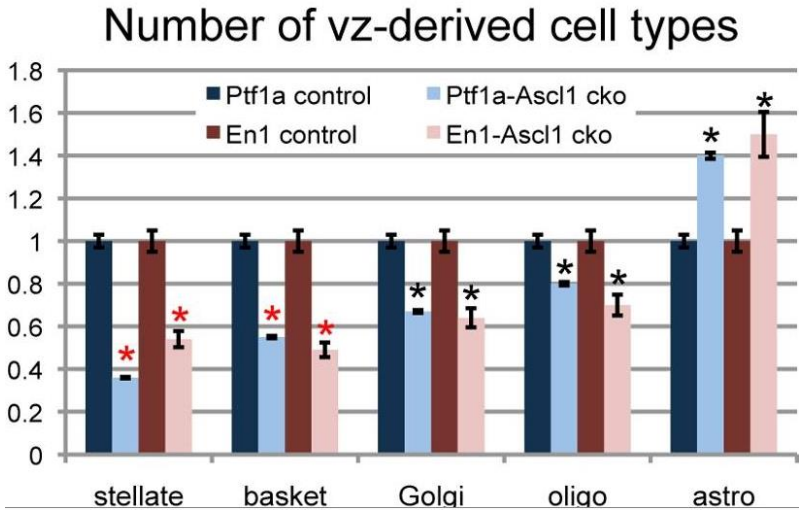
RL: Glutamatergic neurons

Cerebellar embryonic neuroepithelia: The Ventricular Zone(VZ)

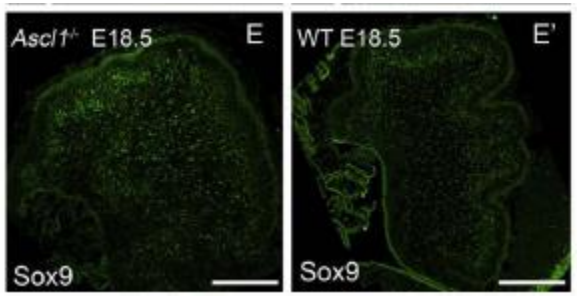


VZ: GABAergic neurons and AS

Ventricular origin of cerebellar astrocytes

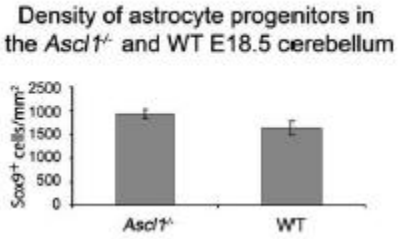


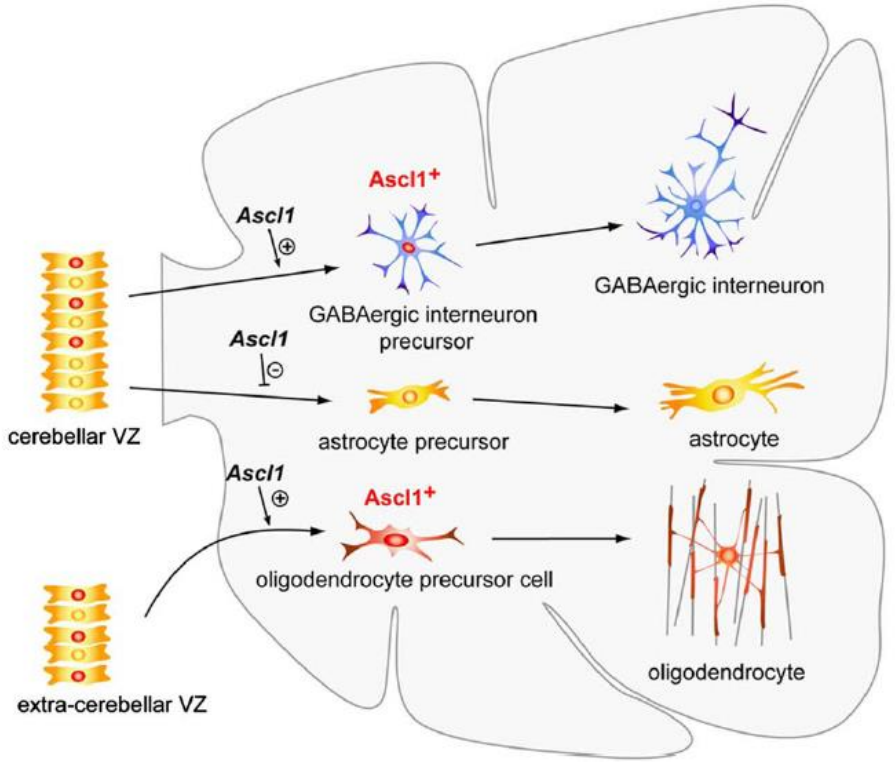
(Sudarov, 2011)



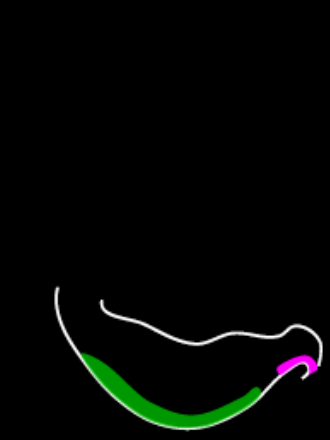
(Grimaldi, 2006)

F

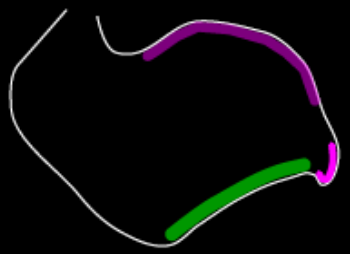




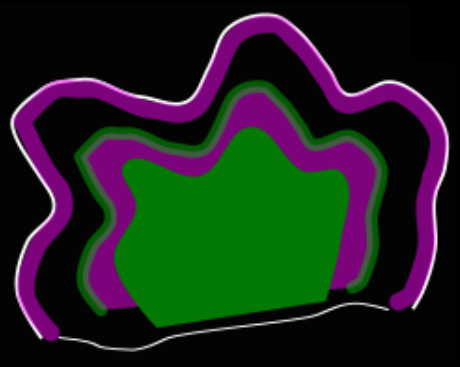
CEREBELLAR DEVELOPMENT



E15

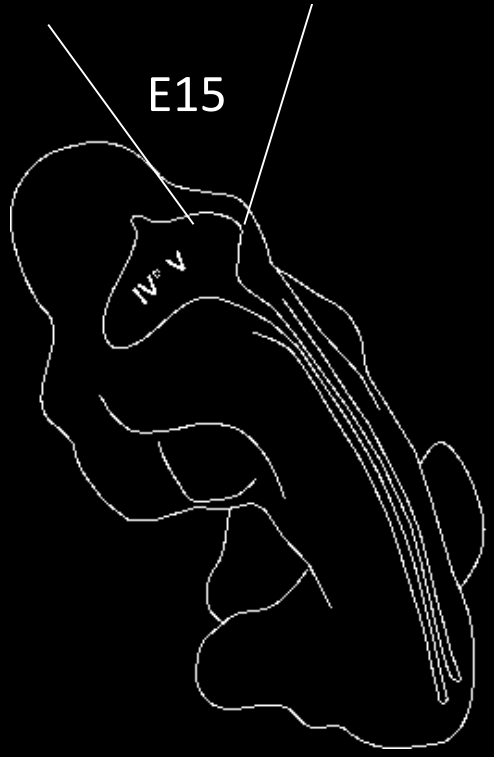


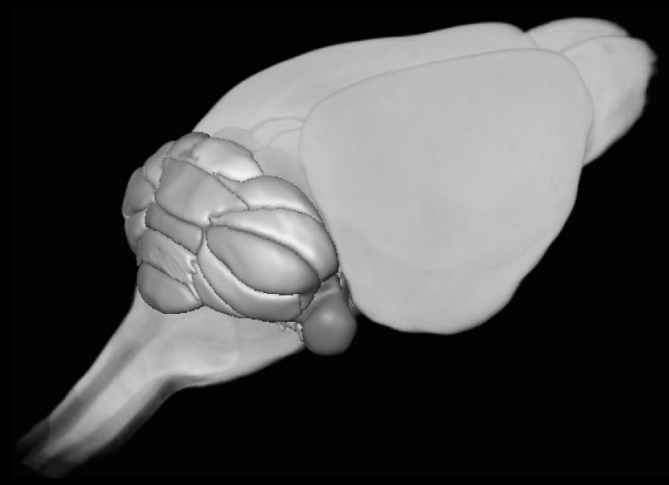
E18



P3

P15



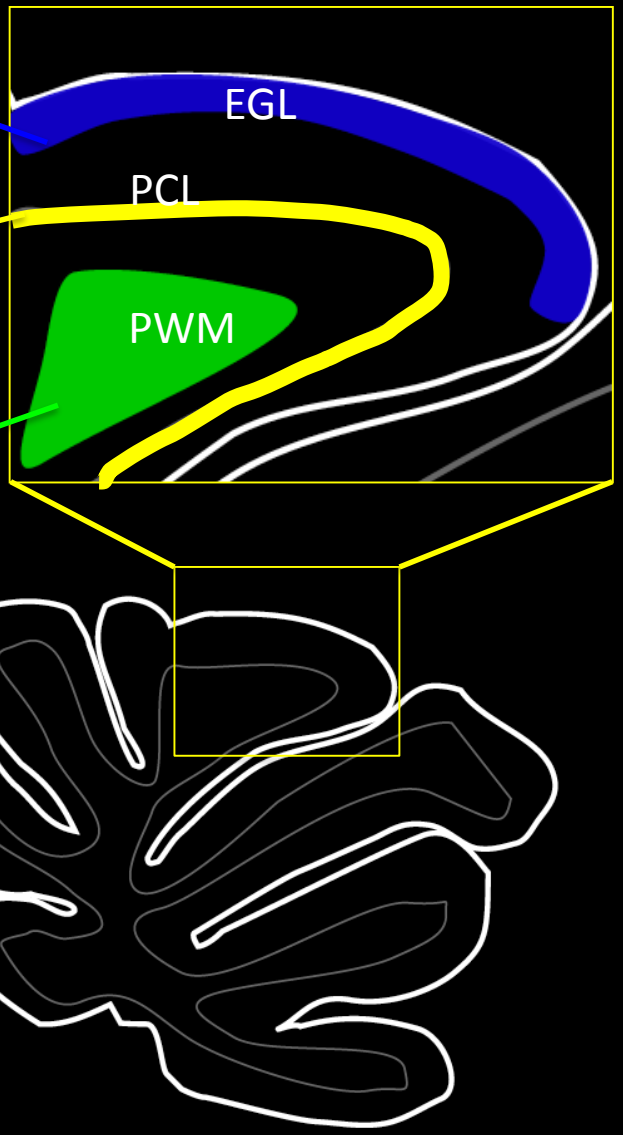


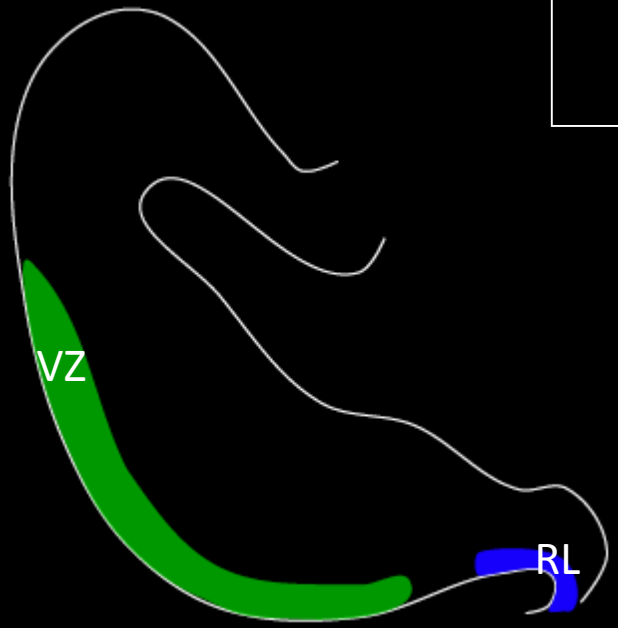
Cerebellar neuroepithelia are maintained after birth

Granule neurons (Glu)

Purkinje cells (GABA) **Astrocytes?**

Interneurons (GABA) **Astrocytes?**

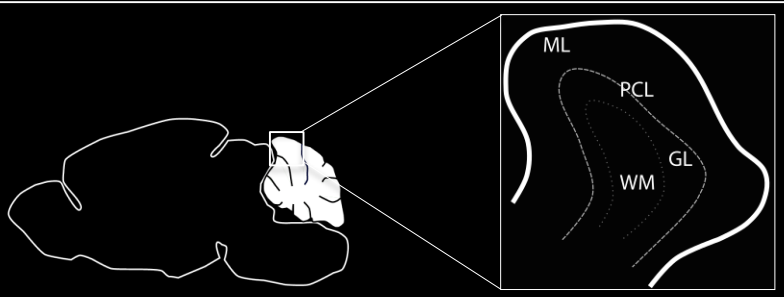




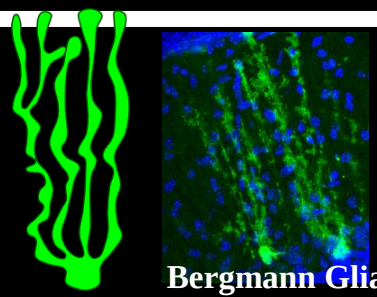
Embryonic development:
primary germinal sites

Postnatal development:
secondary germinal sites

Astroglial phenotypes in the cerebellum

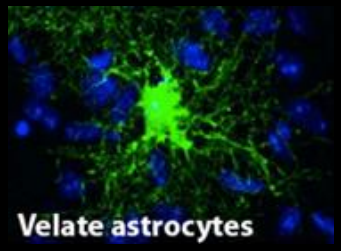


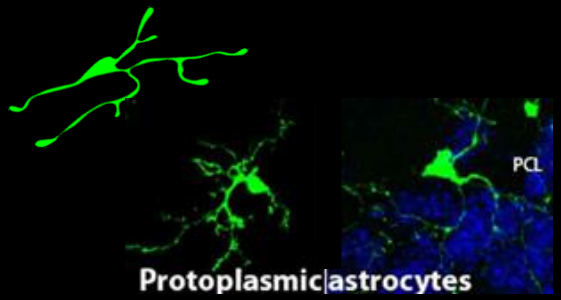
Molecular Layer (ML)



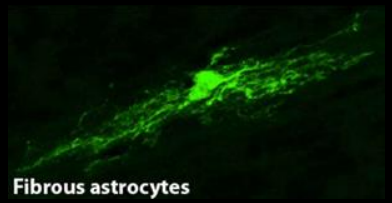
Purkinje Cell Layer (PCL)

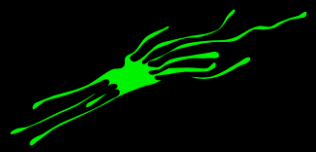
Granular Layer (GL)





White Matter (WM)





Origin of cerebellar astrocytes
QUESTIONS

- Does the variety of astrocytic phenotypes derive from **distinct progenitors**?

OR

- Is it specified from a **single, common ancestor**?

- Are PWM progenitors **multipotent cells** that make their final choice in loco?

OR

- Are they already **fate restricted** when they leave the VZ?

- Does the PCL act as a **secondary gliogenic niche** during postnatal development?

In vivo clonal analysis

…from quail-chick grafts to:

- Regionally-expressed Cre-recombinase lines
- Defective retroviral infections

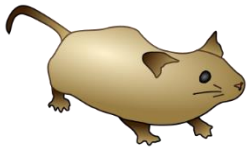
  RV carrying reporter genes (i.e. GFP)
  Retroviral libraries (i.e. QmGFP-OL)

- Mosaic expression of multiple genes

  Brainbow technology
  Confetti mice
  Star-Track plasmids

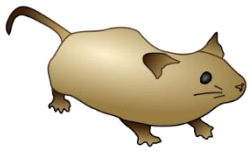
Regionally-expressed Cre-recombinase lines

Cre line



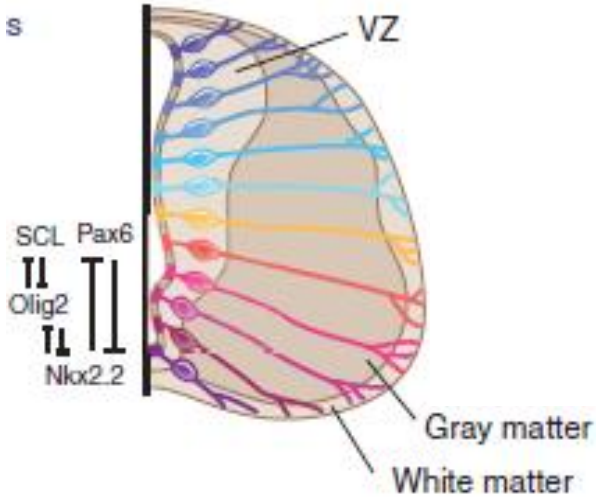
X

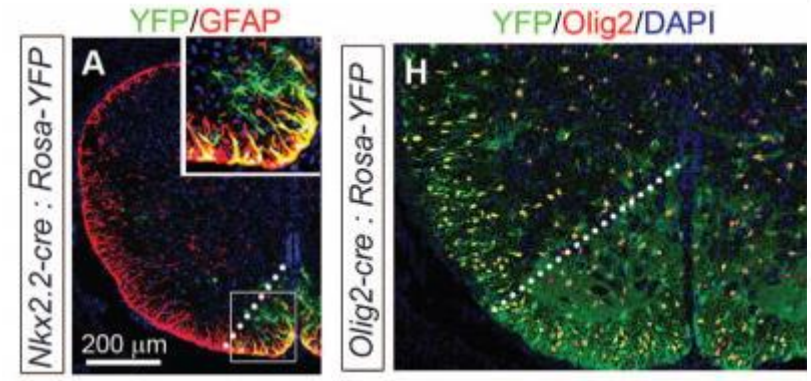
Reporter line

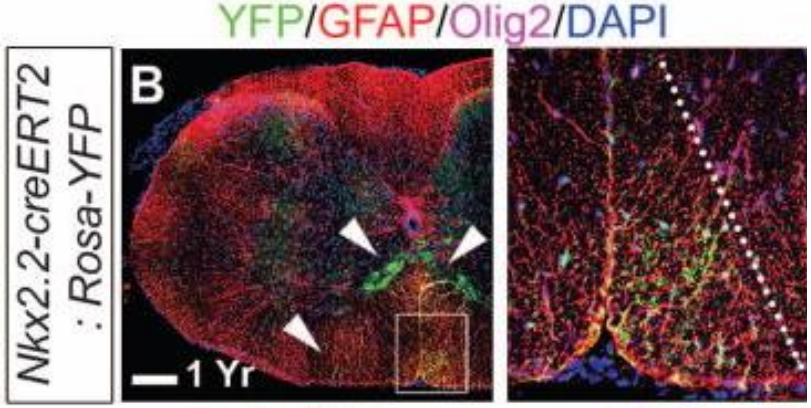


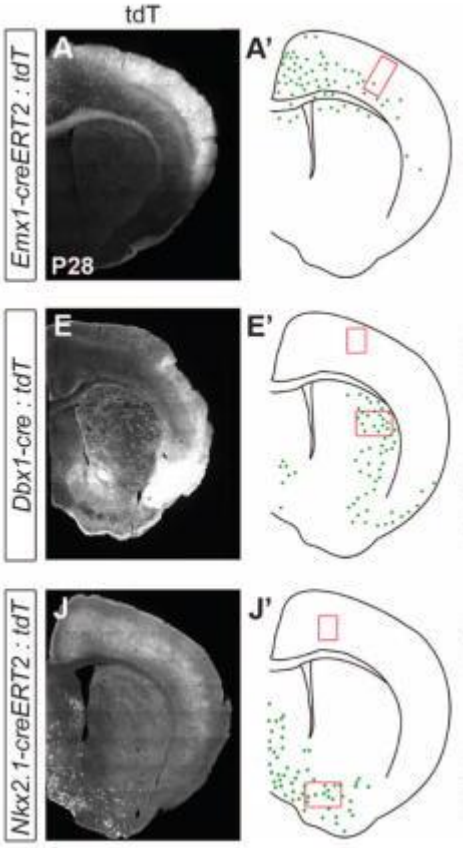
X-Cre
-CreERT2
(i.e.NKx2.2-Cre
-CreERT2)

Rosa-YFP
Rosa26-tdTomato
(…)





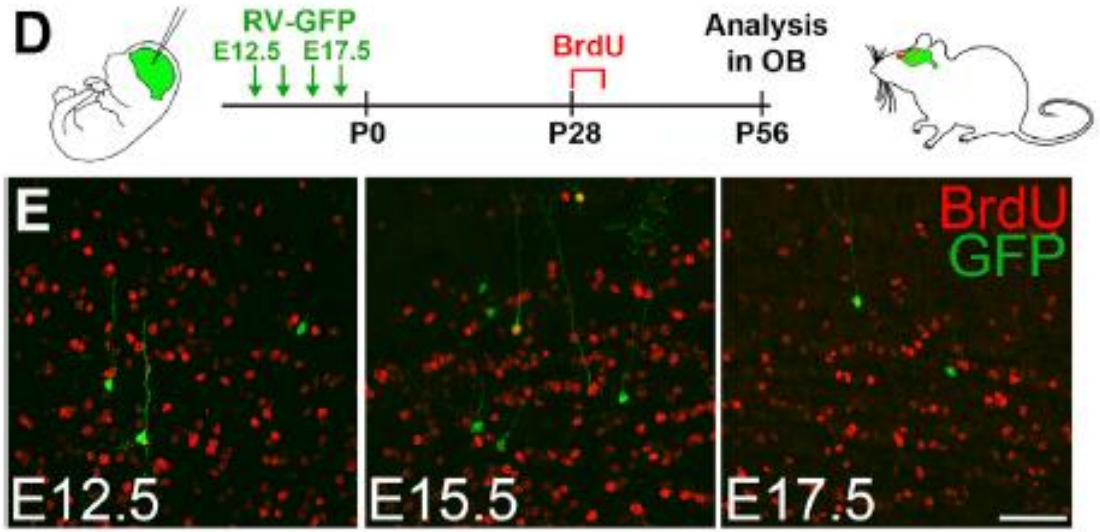




(Tsai, 2012)

Recombinant retrovirus (RRV) infections

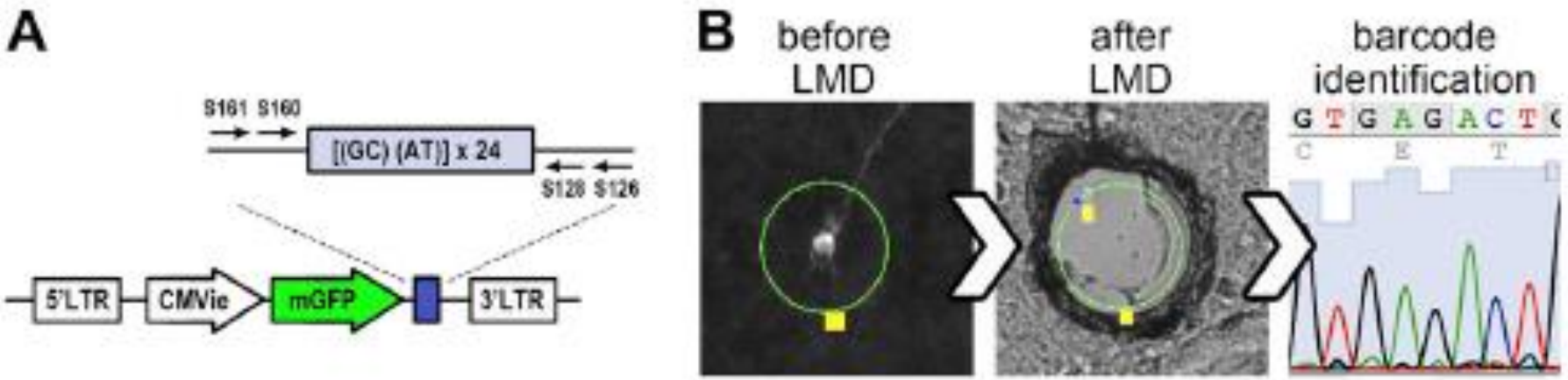
1. RRV carrying **reporter genes**



LIMITATIONS

- The expression depends on its site of integration
- Epigenetic silencing
- Accessibility and infectivity of all cells of the embryo are not uniform (RV only proliferating cells!)

2. **Retroviral libraries**: QmGFP-OL

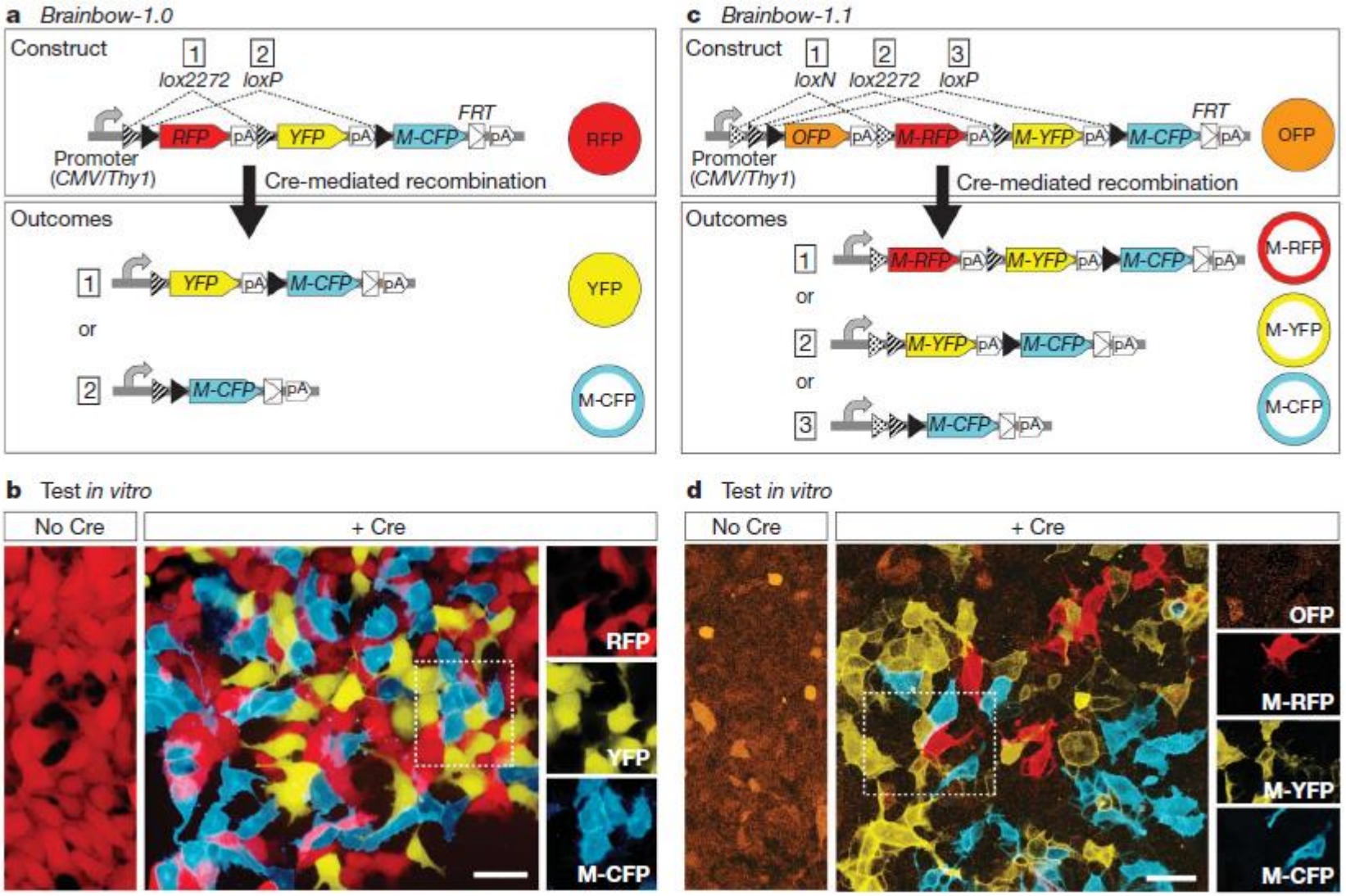


(Fuentealba 2015)

Mosaic expression of multiple genes

1. Brainbow transgenes:

Incompatible sets of lox sites

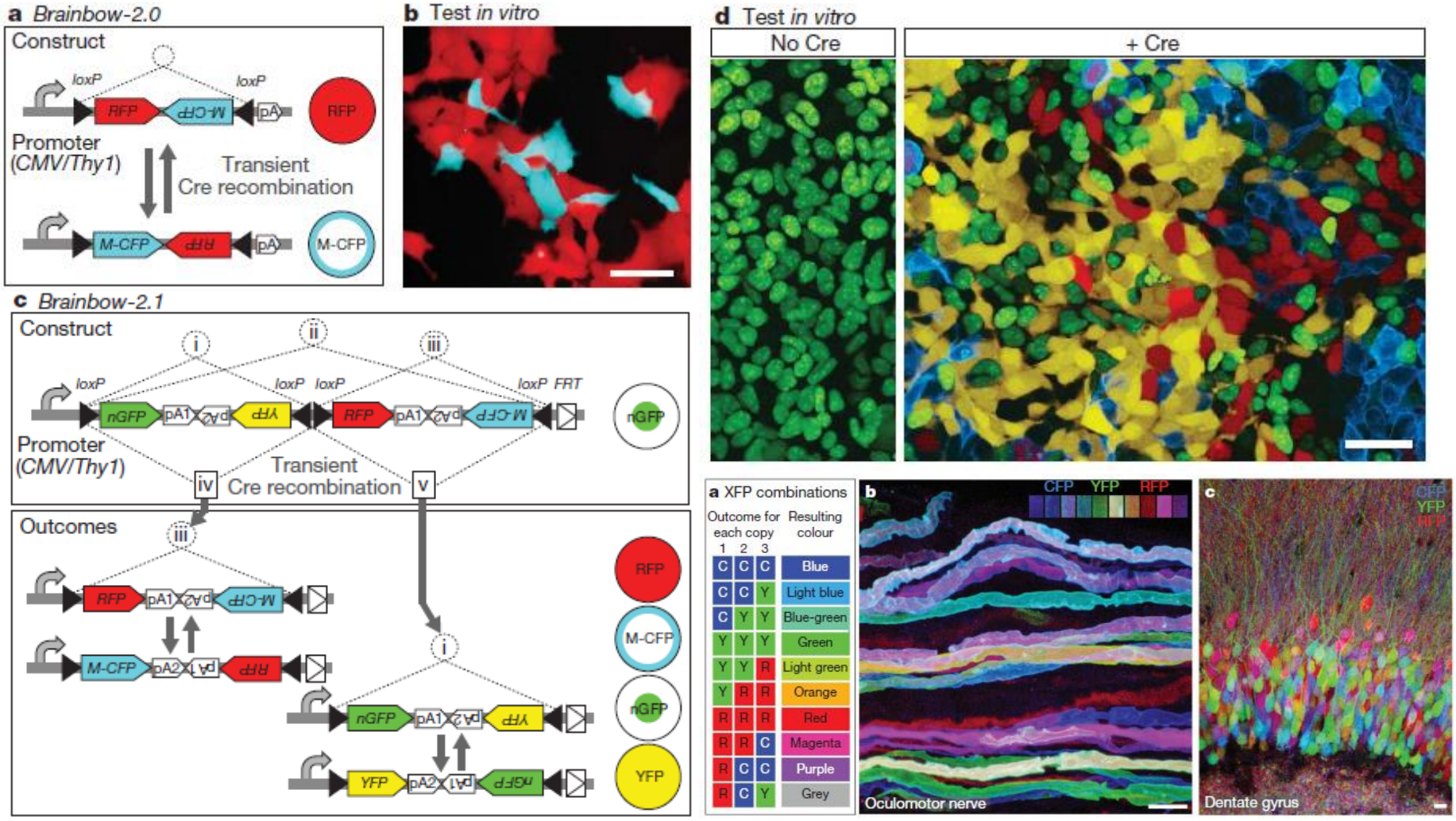


(Livet, 2007)

Mosaic expression of multiple genes

1. Brainbow transgenes:

Cre-mediated inversion

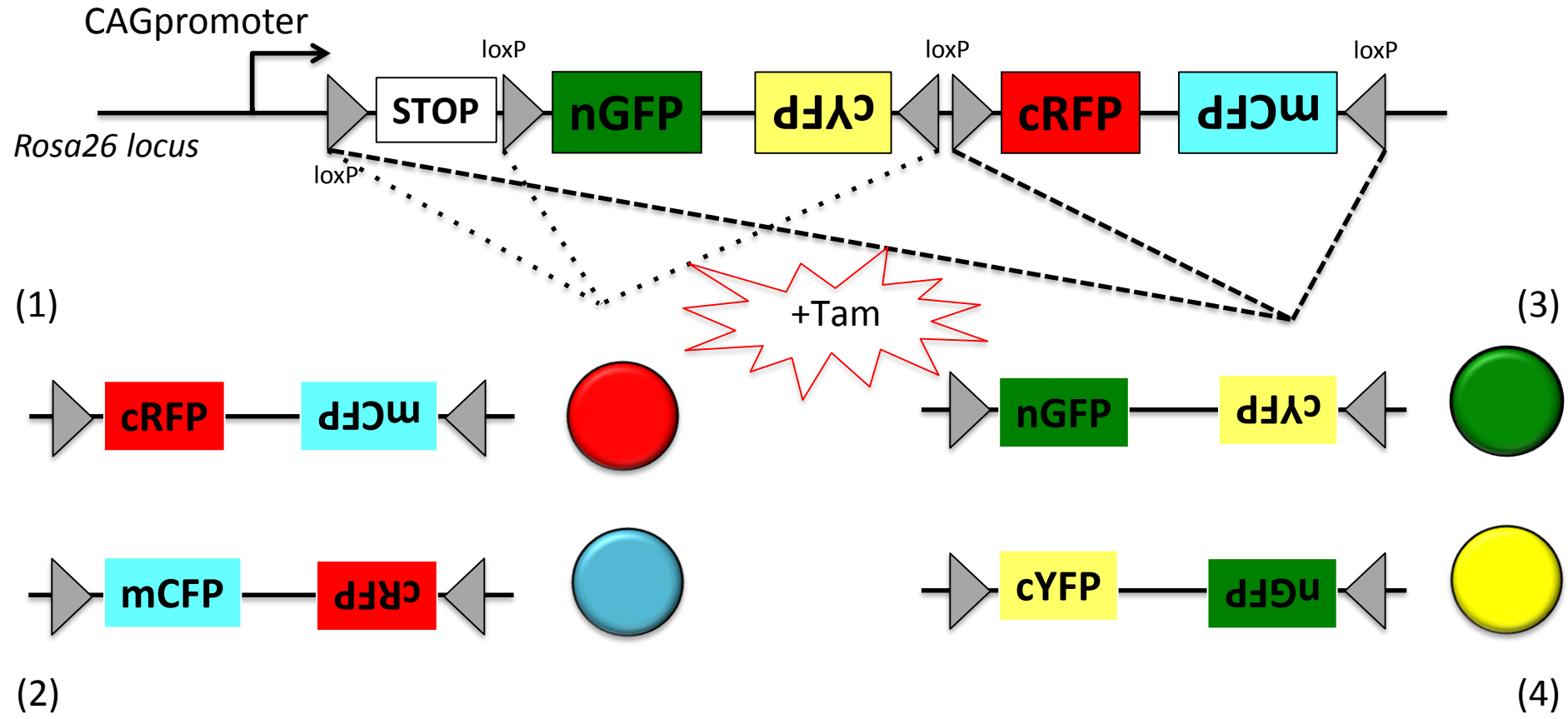


(Livet, 2007)

Mosaic expression of multiple genes

2. Confetti mice

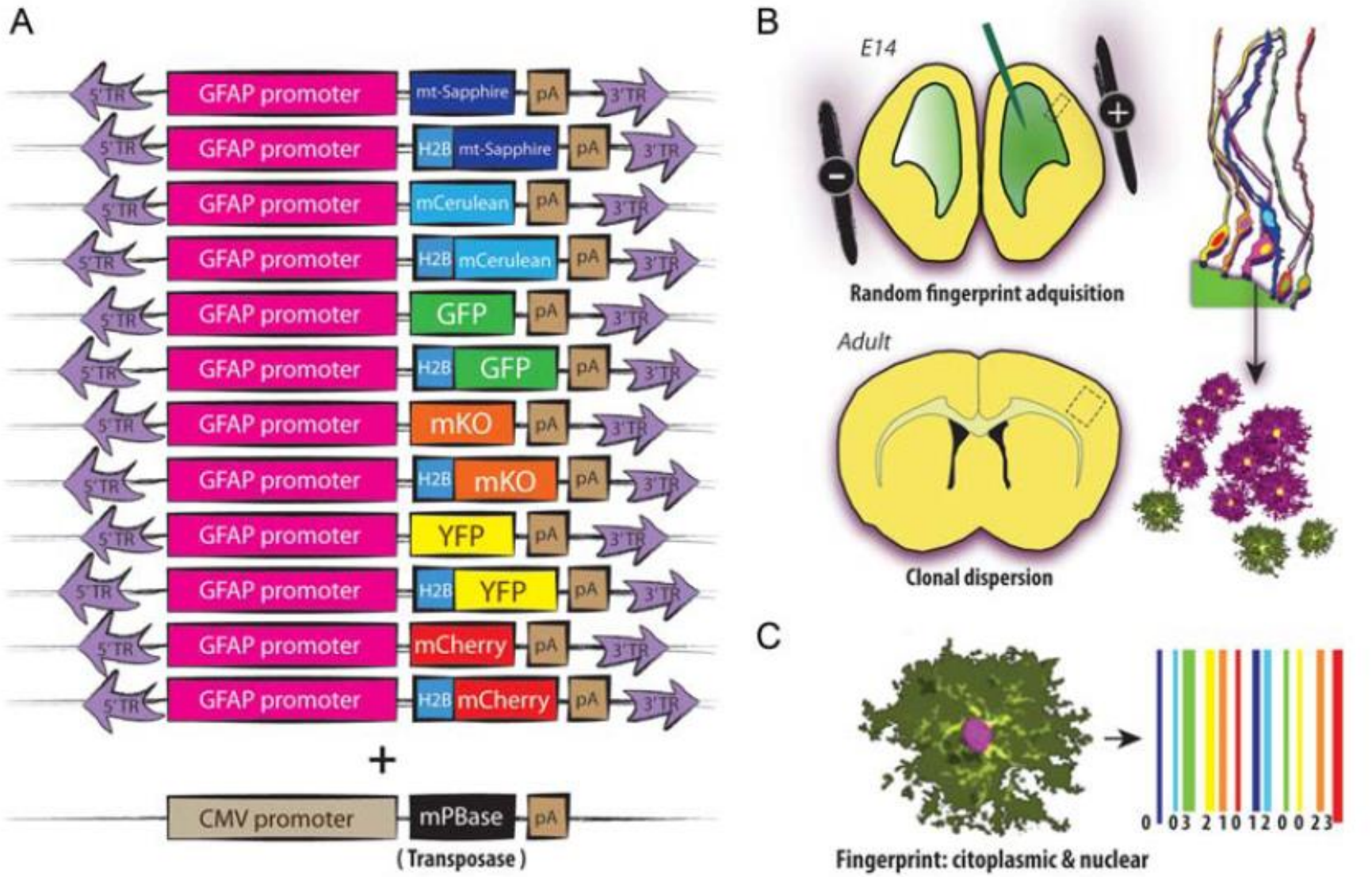
Brainbow 2.1



(Snippert et al, 2010)

Mosaic expression of multiple genes

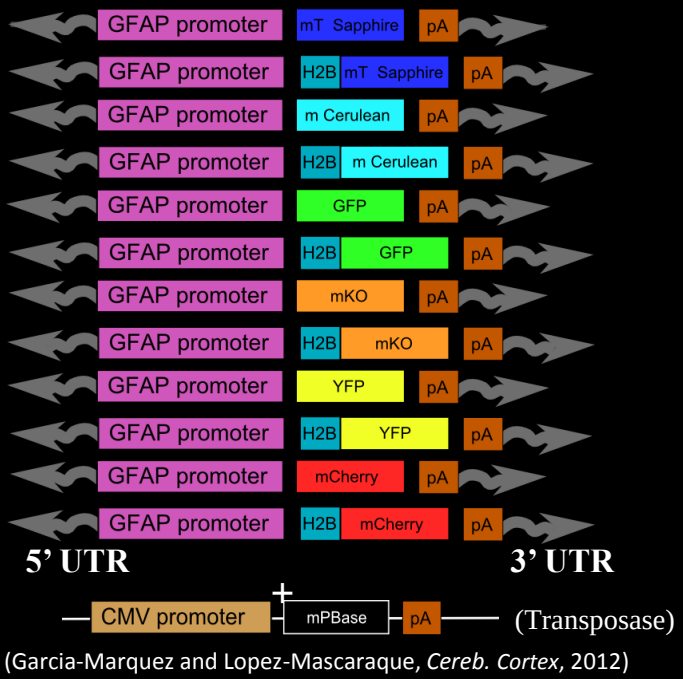
3. Star-Track plasmids



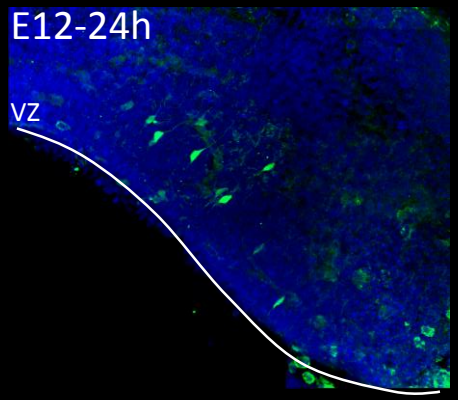
(Garcia-Marques and Lopez-Mascaraque, *Cereb. Cortex*, 2012)

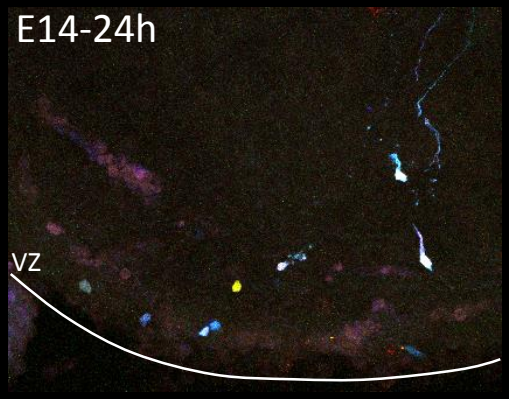
HOW IS THE ASTROGLIAL HETEROGENEITY GENERATED?

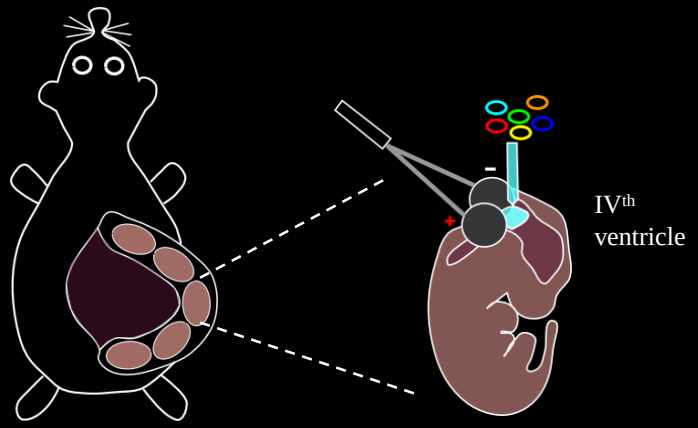
In vivo clonal analysis: Star Track

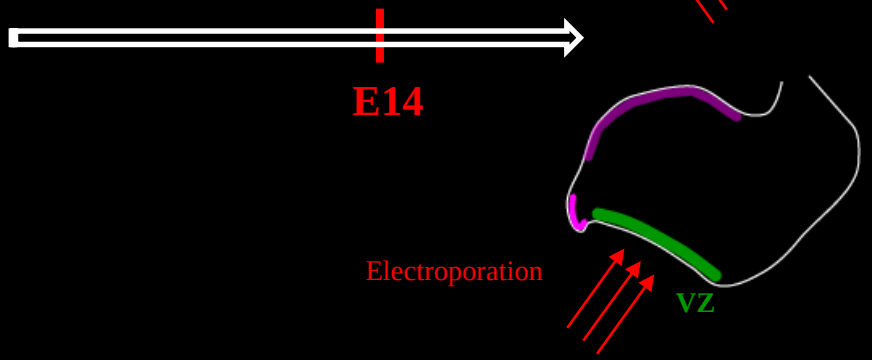


(Garcia-Marquez and Lopez-Mascaraque, *Cereb. Cortex*, 2012)

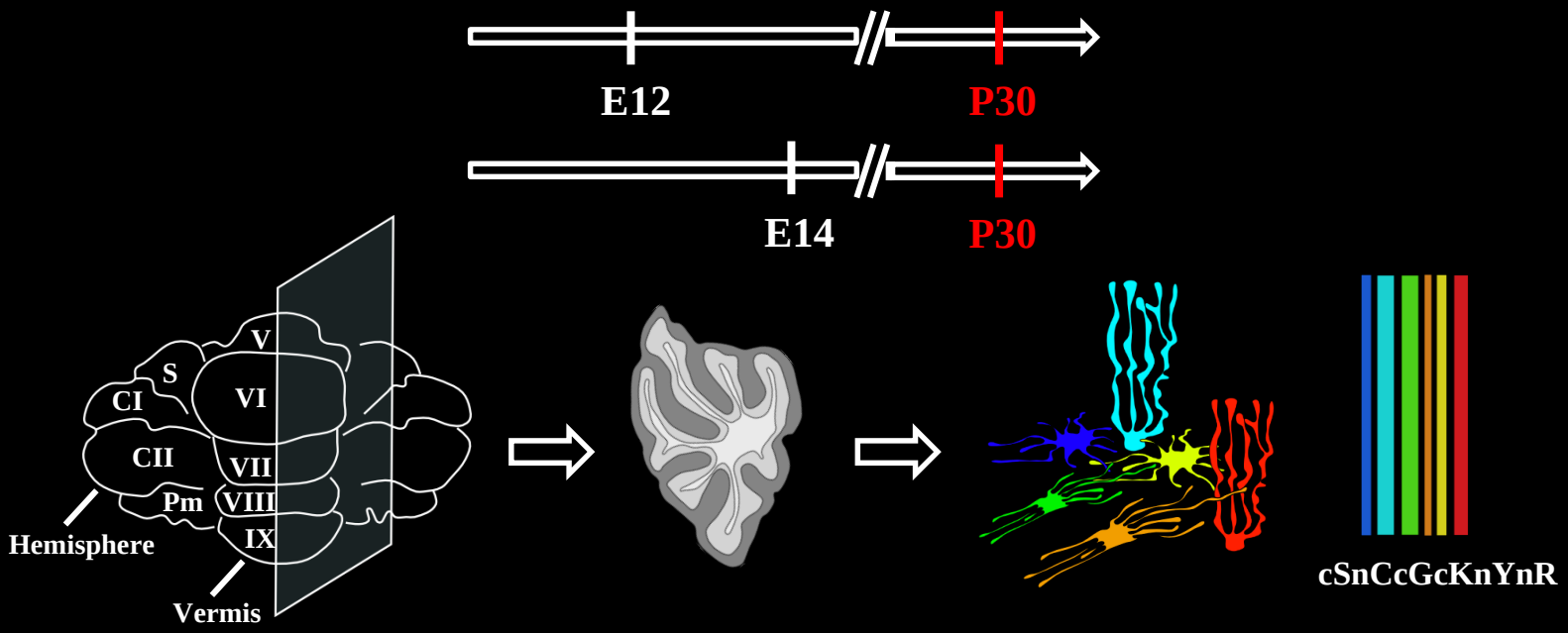


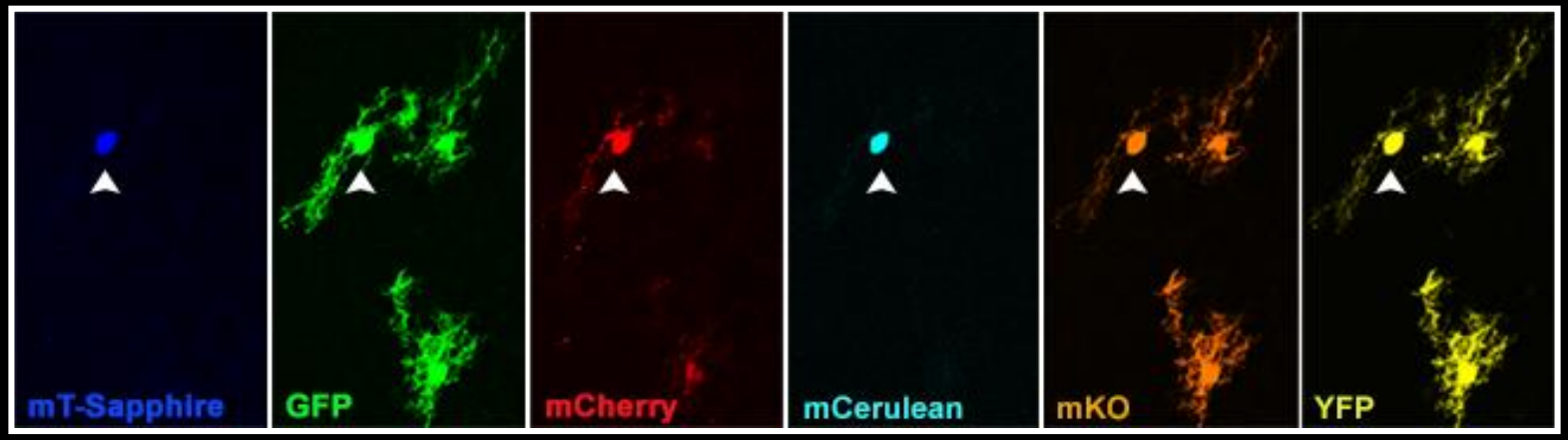




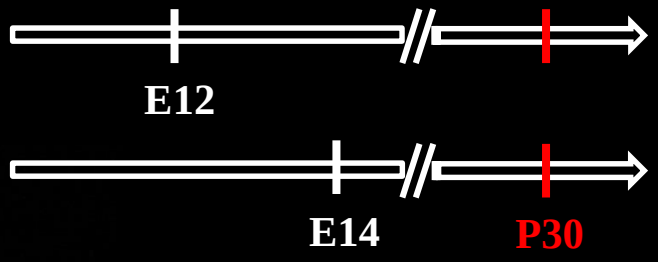


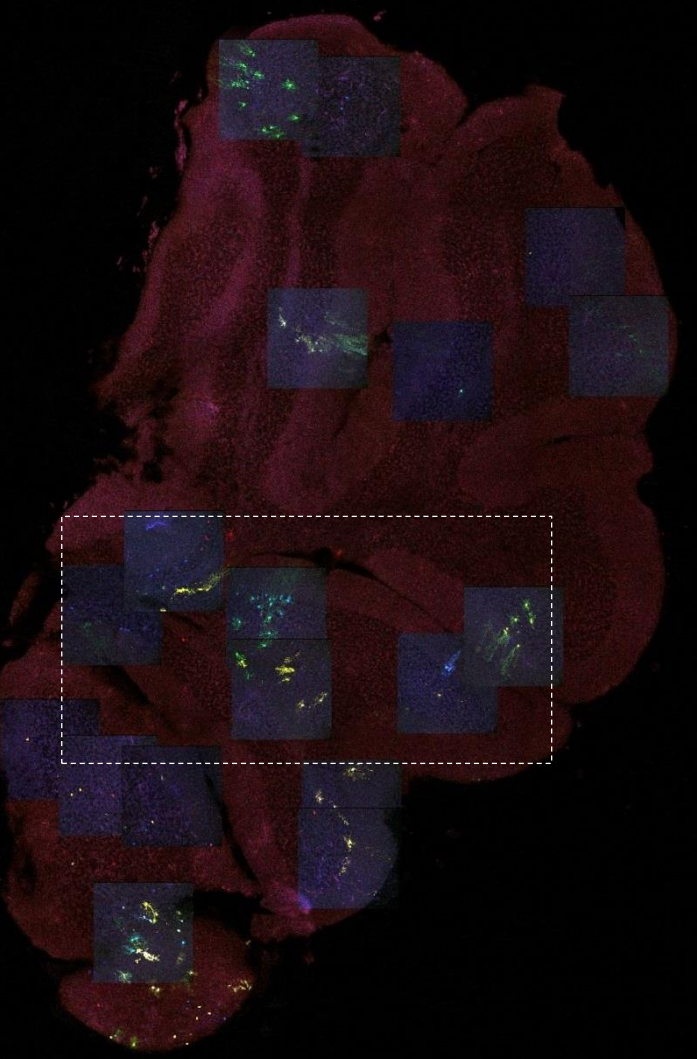
In vivo clonal analysis: Star Track

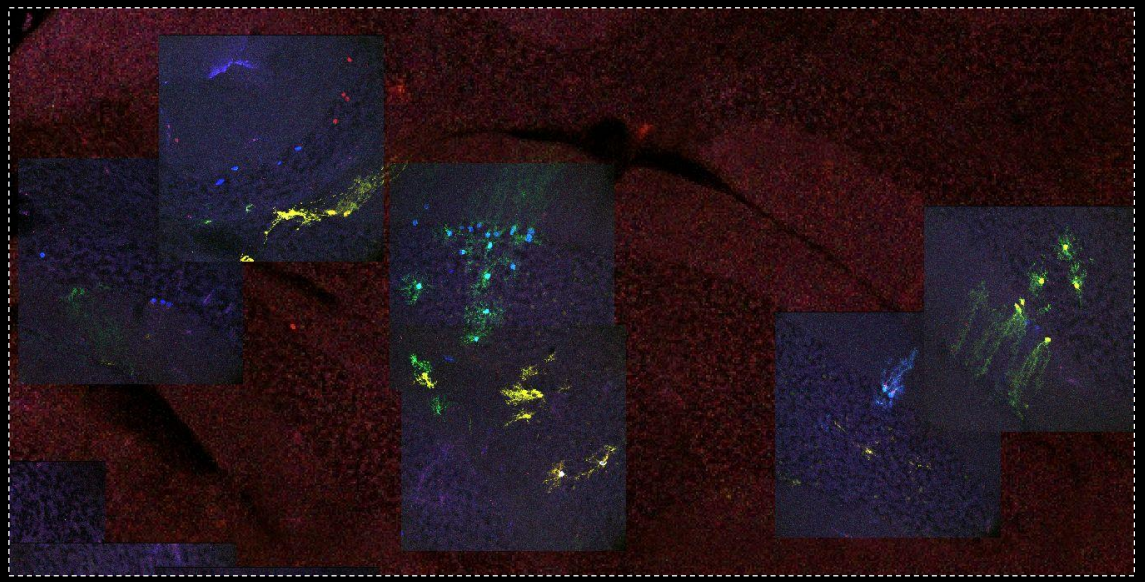




In vivo clonal analysis: Star Track



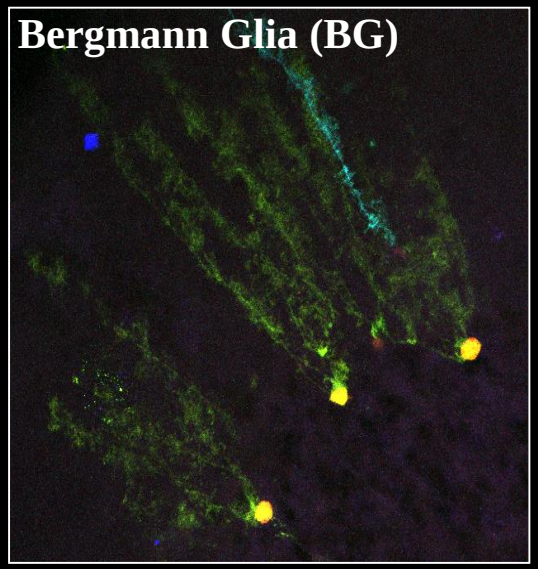


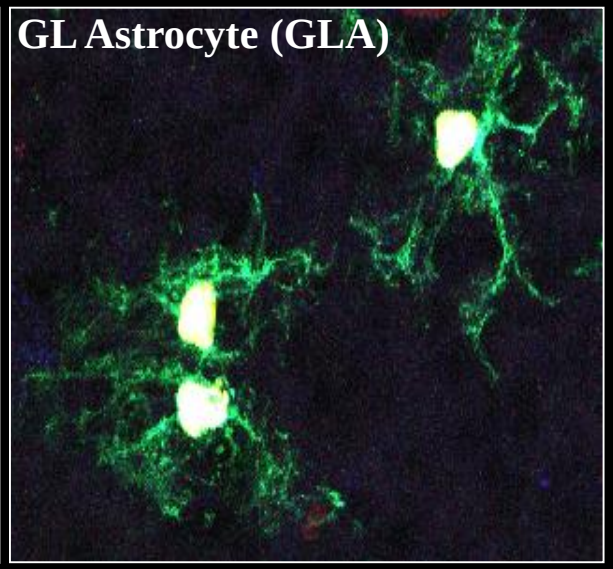


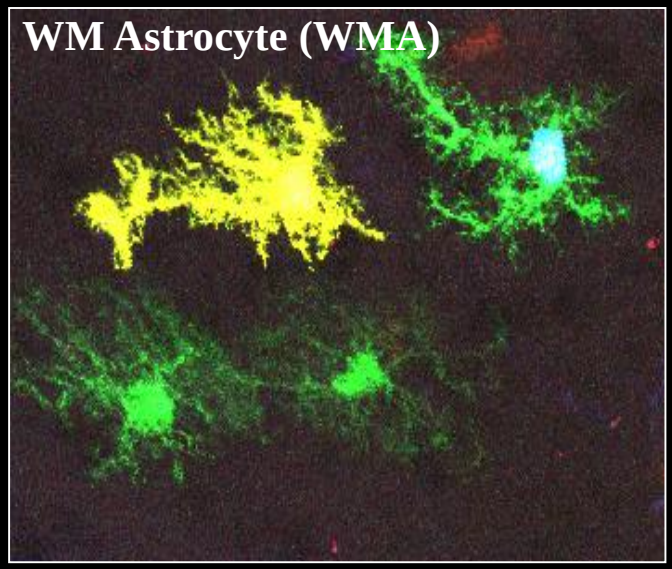
20X → 40X

In vivo clonal analysis: Star Track

Clones defined by **localization and morphology**





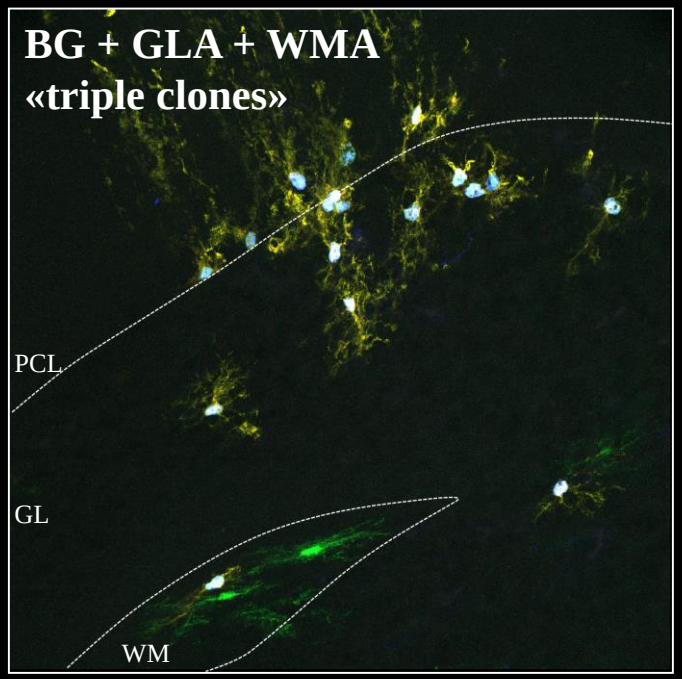


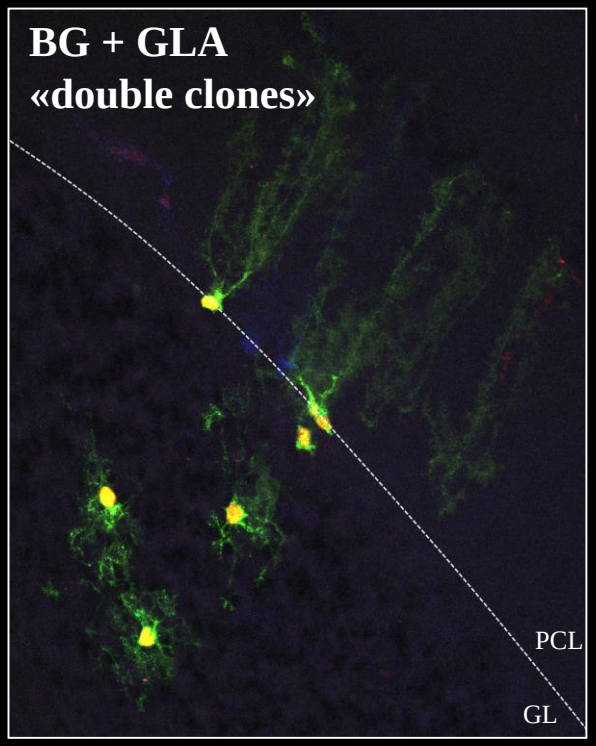
HOMOGENEOUS CLONES (HomCs)

Clones composed of astrocytes of the same morphology and layering

In vivo clonal analysis: Star Track

Clones defined by **localization and morphology**

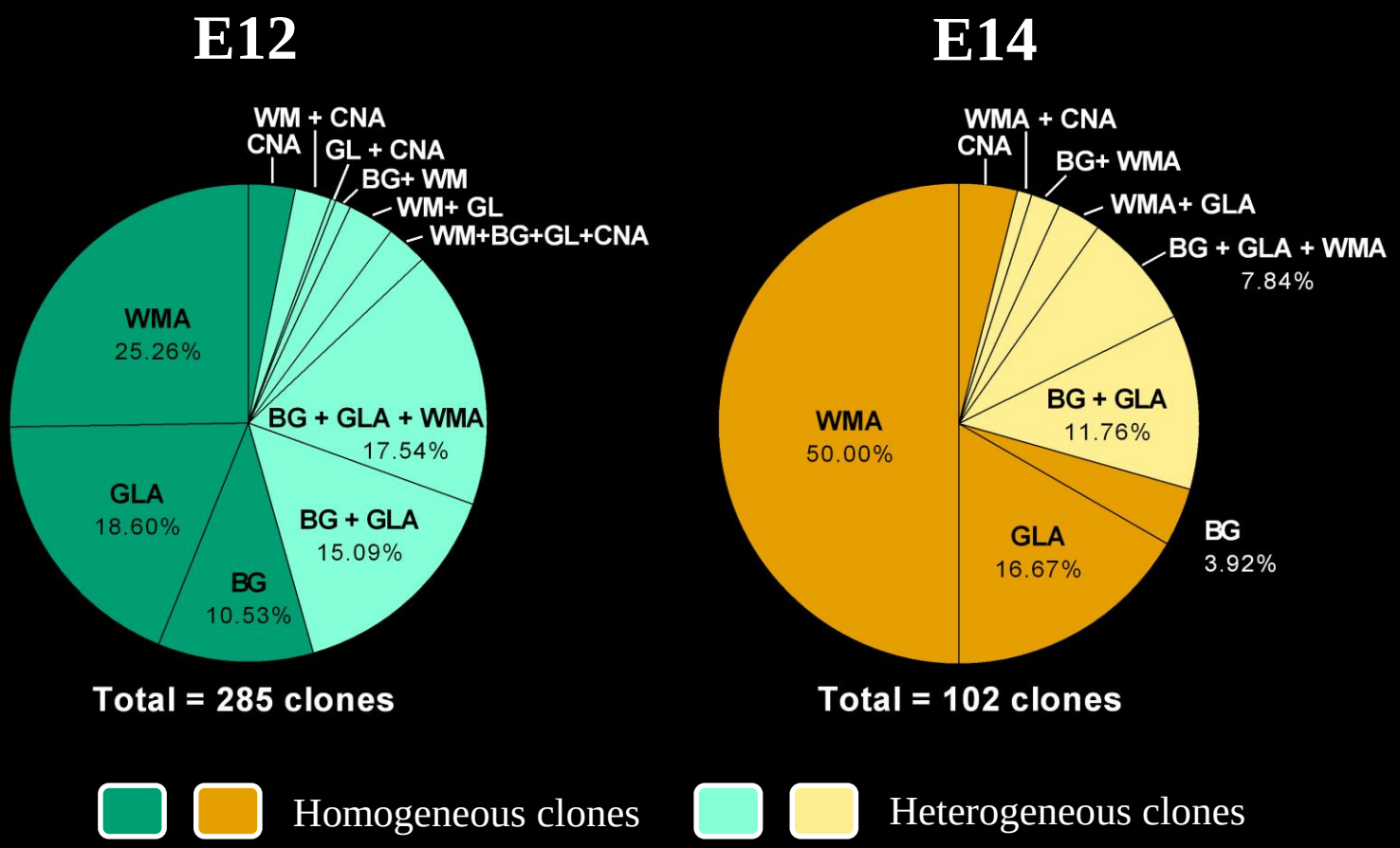




HETEROGENEOUS CLONES (HetCs)

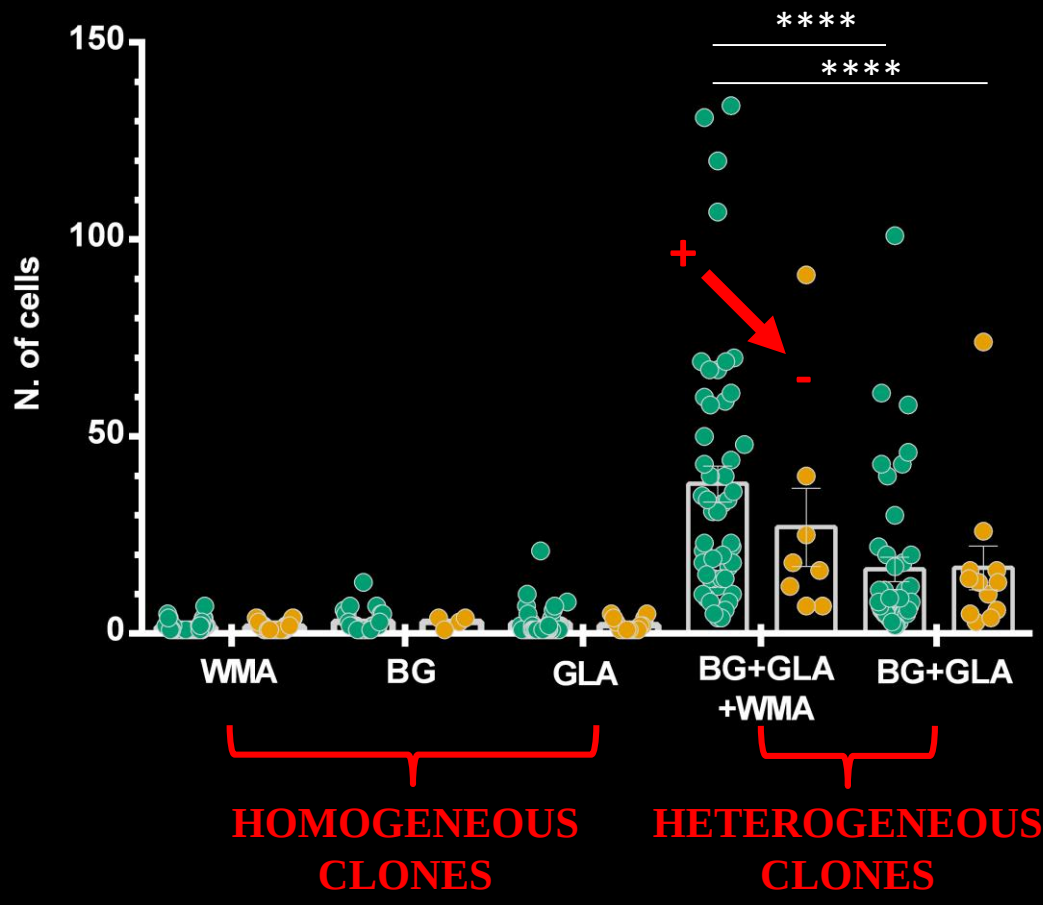
Clones including cells with various phenotypes spreading over different layers

CLONE COMPOSITION



Tracking astrogliogenesis at distinct embryonic stages reveals a developmental shift from multipotent to fate-restricted progenitors

CLONE SIZE



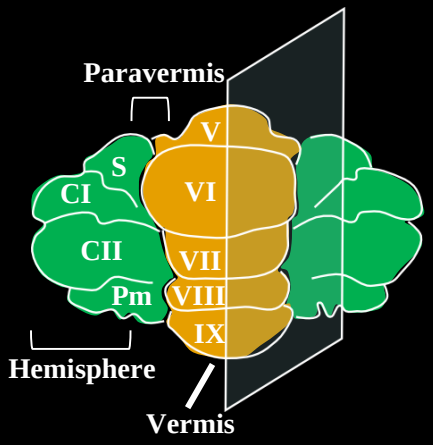
E12 – P30

E14 – P30

*** P<0.0001

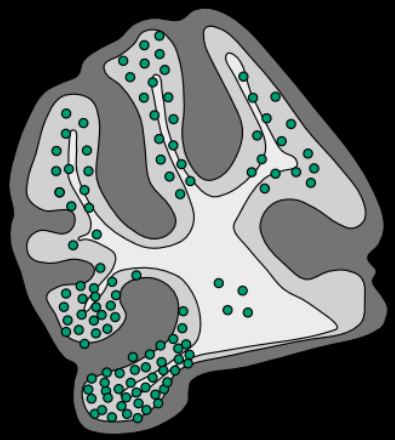
The size of clones increases in parallel with their degree of heterogeneity and tends to decrease with time

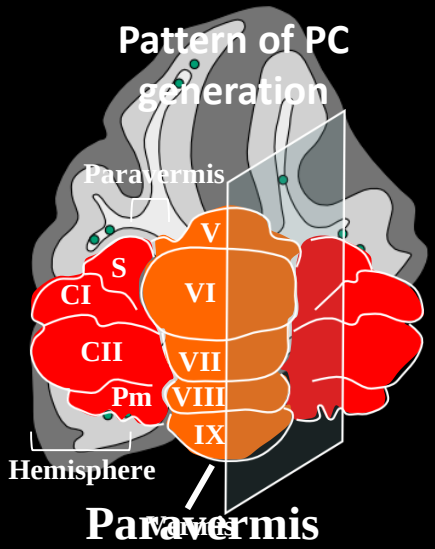
CLONE DISPERSION

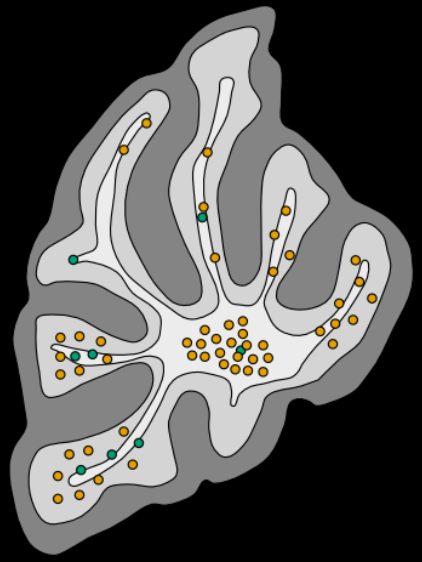


Early (E12) and late (E14) progenitors generate spatially restricted astrocytes along the Medio-Lateral axis

TEMPORAL PATTERN → SPATIAL PATTERN





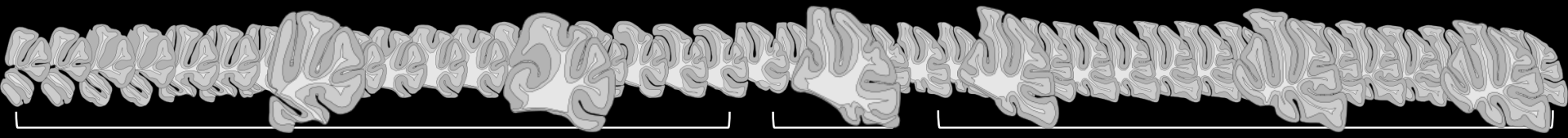


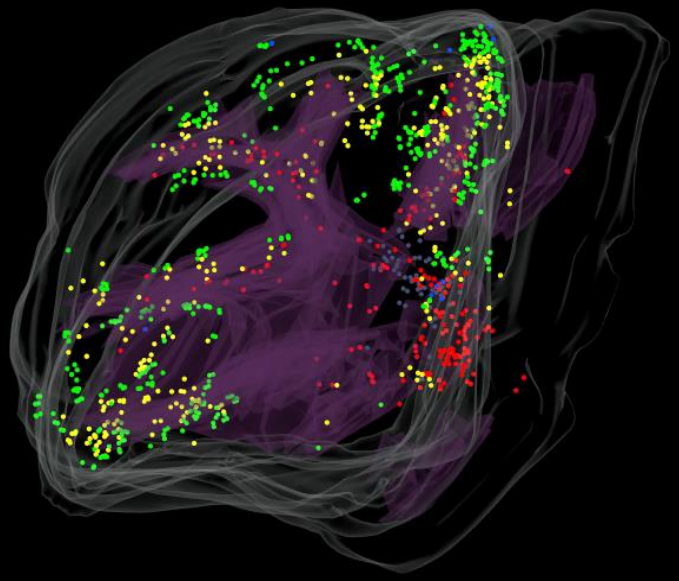
Hemisphere Paravermis Vermis

E12 – P30 E14 – P30

CLONE DISPERSION

Homogeneous Clones





GLA clone
E12-P30

WMA Clone
E14-P30

BG Clone
E12-P30

GLA Clone
E14-P30

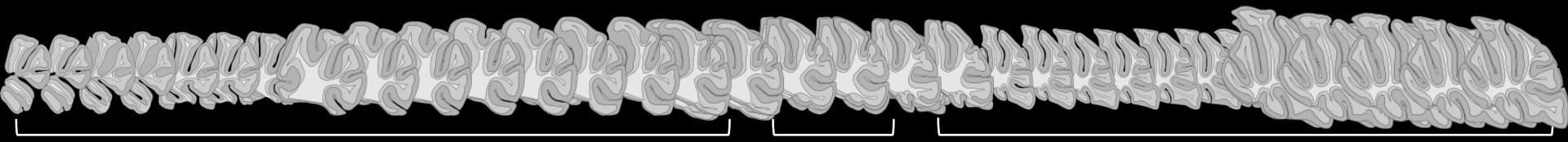
WMA clone
E12-P30

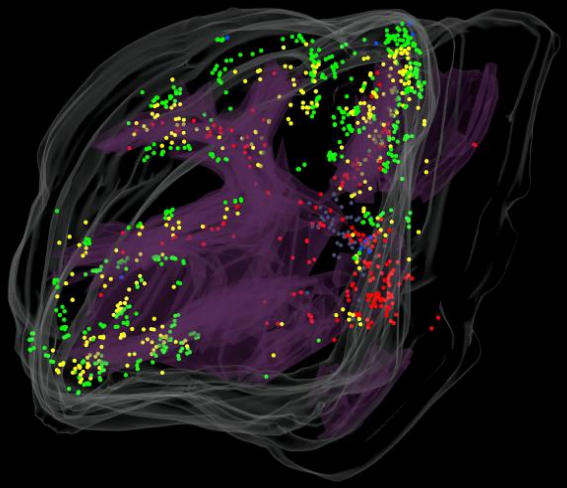
BG Clone
E14-P30

The majority of **Homogeneous Clones** are restricted to a single cerebellar section

CLONE DISPERSION

Heterogeneous Clones





BG+GLA Clone
E12-P30

BG+GLA Clone
E14-P30

BG+GLA+WMA Clone
E12-P30

BG+GLA+WMA Clone
E14-P30

Heterogeneous Clones appear much more dispersed along the medio-lateral axis, especially those generated from early (E12) progenitors

In vivo clonal analysis: Star Track

CONCLUSIONS – Part 1 -

- Astroglial heterogeneity is generated according to a well defined **temporo-spatial patterning : time** defines the fate of progenitor cells and the final allocation of their progenies

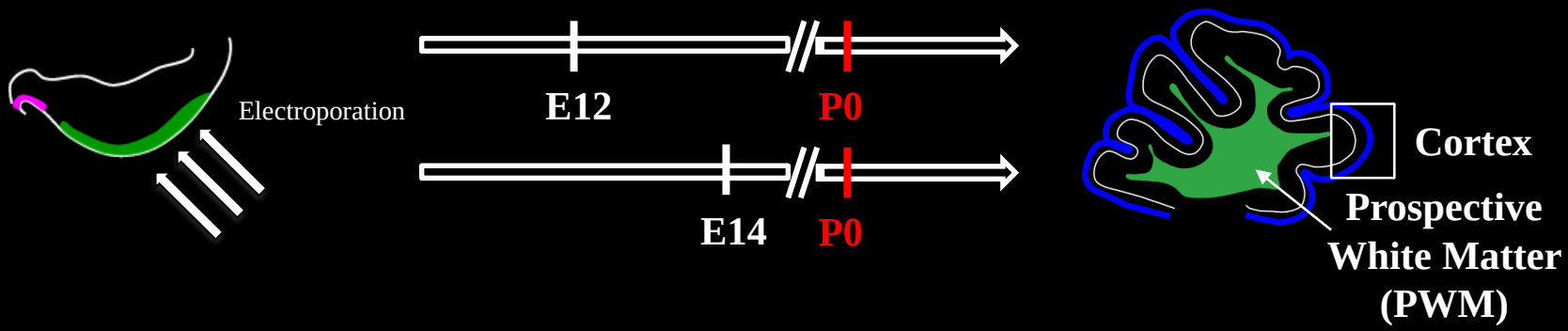
- The degree of heterogeneity of clones strictly influences both their size and dispersion

MORE CELLS → MORE DISPERSION

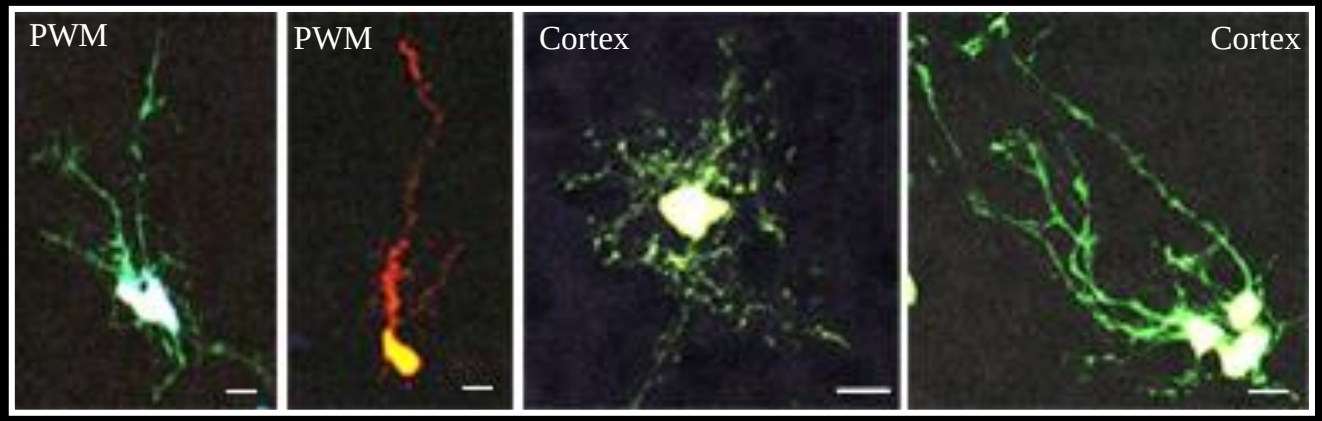
QUESTION

When do the distinct fates and features of clones emerge?

In vivo clonal analysis: Star Track

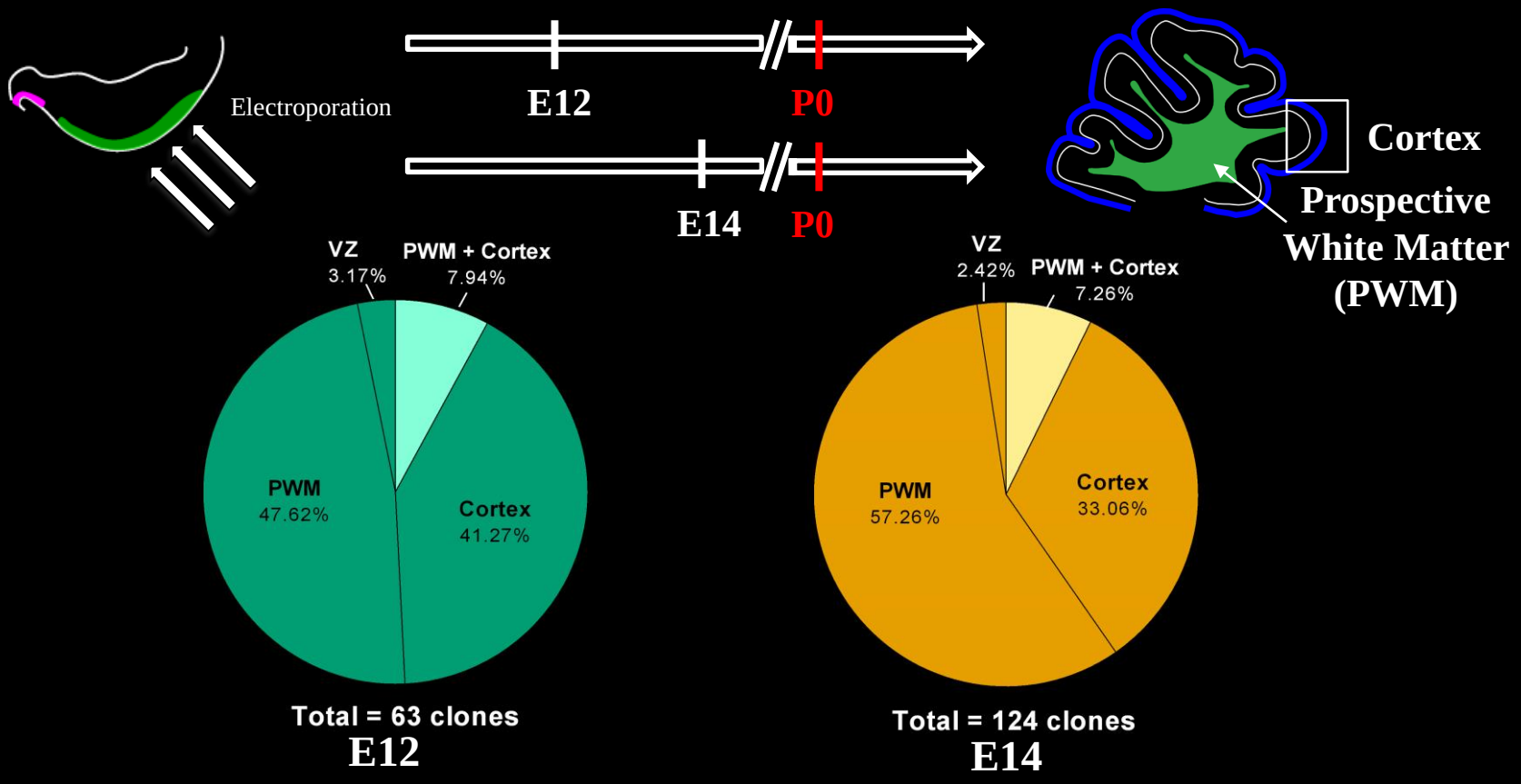


Clones defined
by **localization**



Scale bars: 10μm

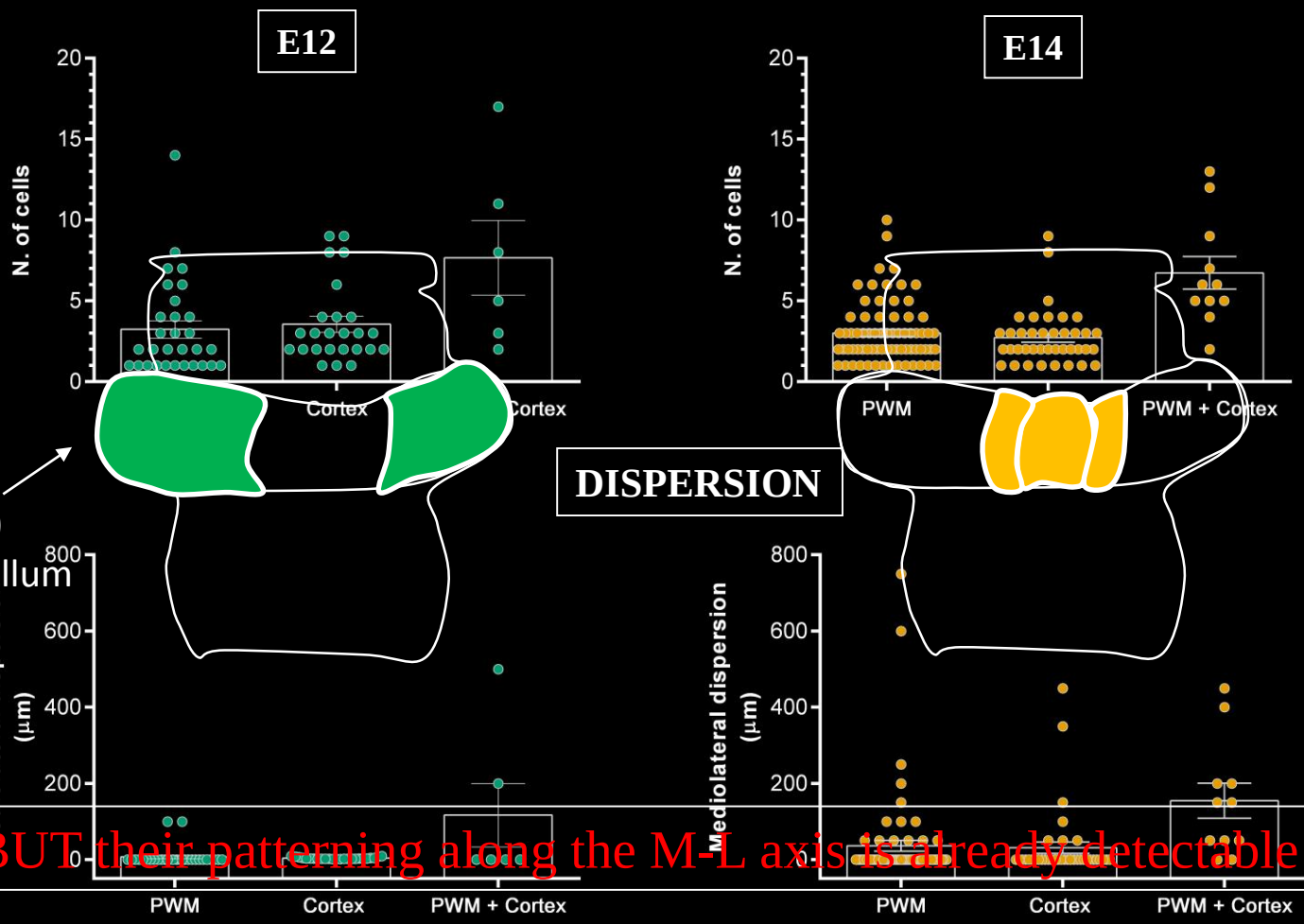
In vivo clonal analysis: Star Track



At birth, astrocytic progenies born at different embryonic ages are equally distributed in the cerebellar layers

Heterogeneous clones have still barely emerged

M-L ORGANIZATION



P0 cerebellum

BUT their patterning along the M-L axis is already detectable at P0

They are also very similar in term of size and degree of dispersion

In vivo clonal analysis: Star Track

CONCLUSIONS – Part 2 -

- The different **fates** and features (size and dispersion) of clones will emerge **during postnatal development**

- **The spatial pattern is already defined early postnatally**

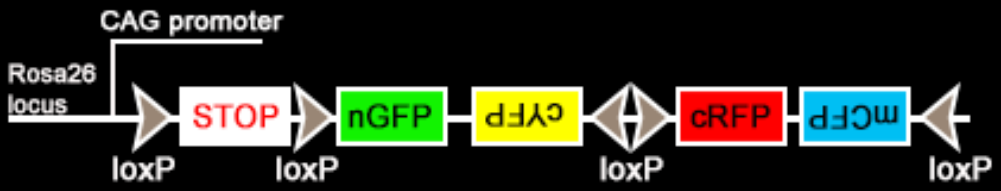
QUESTION

- Clusters of BG and GLA are often found associated in close proximity
- The numbers of BG and GLA are directly correlated
- The numbers of BG exceed that of GLA

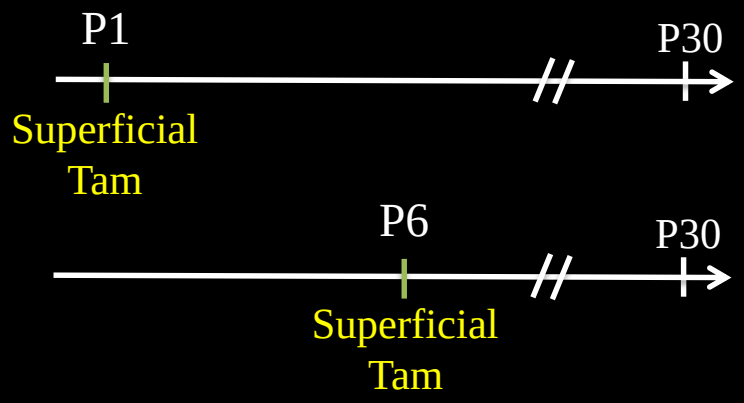
May developing BG act as multipotent progenitors?

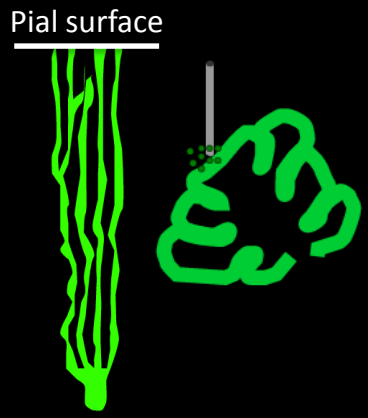
Fate mapping of Glast^{+} BG progenitors

Confetti mice



R26R^{Confetti} X GLAST :: CreER^{T2}

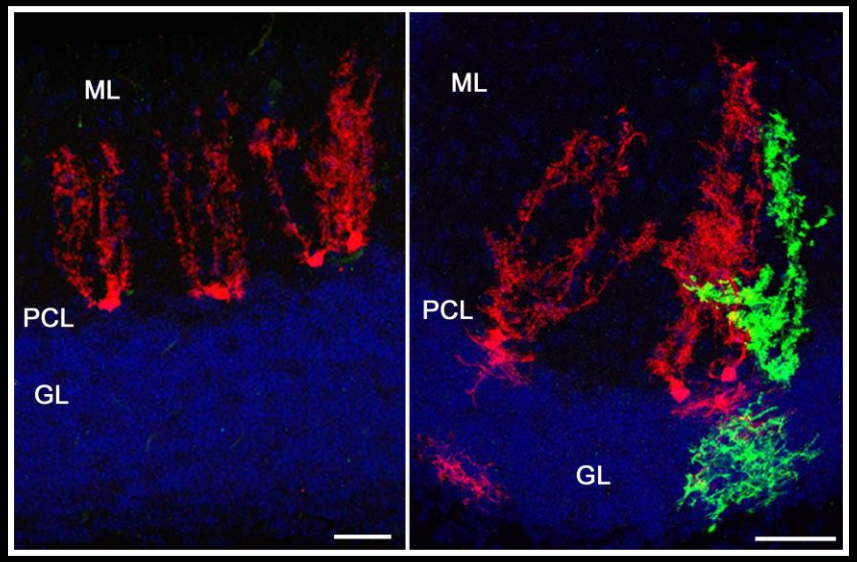


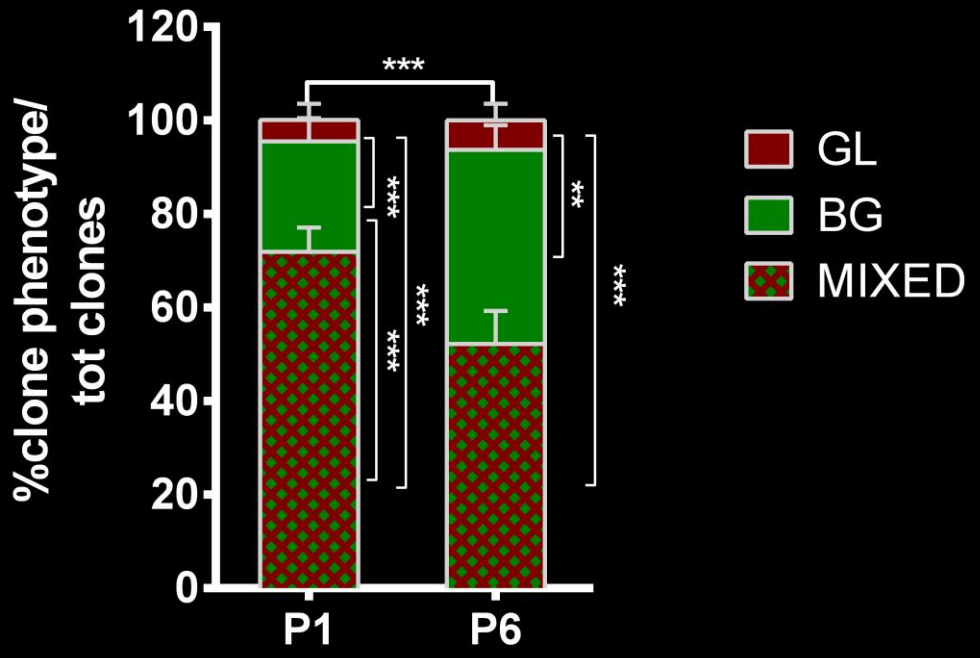


(Parmigiani et al., *J Neurosc,* 2015)

Fate mapping of Glast^{+} BG progenitors

Confetti mice





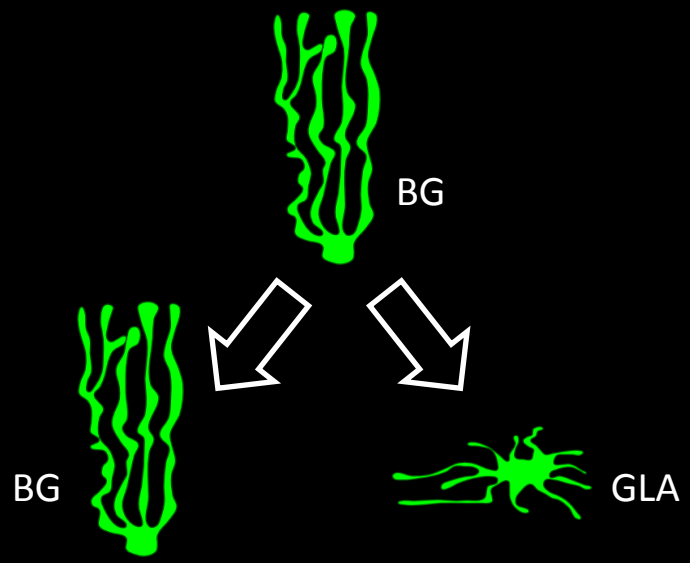
BG can generate astrocytes of the GL during postnatal development

Fate mapping of Glast^{+} BG progenitors

CONCLUSIONS – Part 4 -

- PCL acts as a **SECONDARY GLIOGENIC NICHE,** where BG progenitors produce *in situ* part of GL astrocytes.

- This is likely to be a source for the double BG+GLA clones observed in the *in vivo* Star Track clonal analysis.



CONCLUSIONS