



**UNIVERSITÀ
DEGLI STUDI
DI TRIESTE**



Dipartimento di
Scienze della Vita

COMPARATIVE BRAIN EVOLUTION

Comparative Histogenesis (I)

Lecture 7: Comparative Histogenesis (I)

1. Comparative aspects of cortical neurogenesis in vertebrates

2. Pallial neurogenesis in sharks

J. Anat. (2007) **211**, pp164–176

doi: 10.1111/j.1469-7580.2007.00769.x

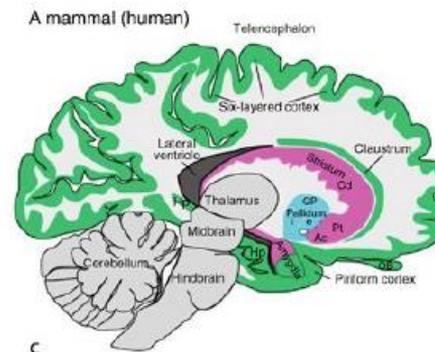
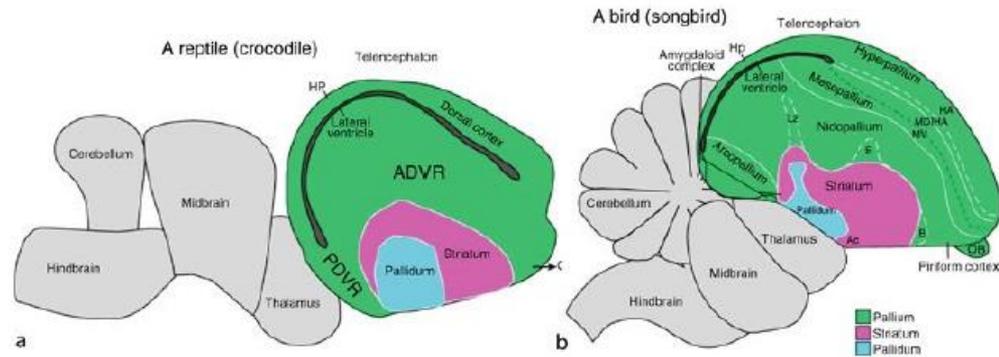
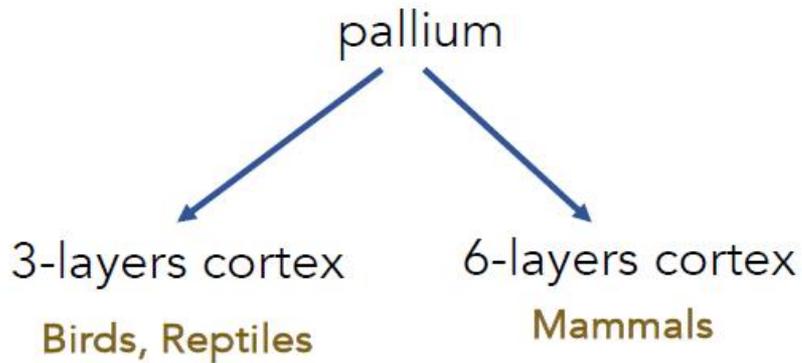
REVIEW

Comparative aspects of cortical neurogenesis in vertebrates

Amanda F. P. Cheung, Alexander A. Pollen, Aniket Tavare, Jamin DeProto and Zoltán Molnár

Department of Physiology, Anatomy and Genetics, University of Oxford, UK

Elaboration of the telencephalon in amniote evolution



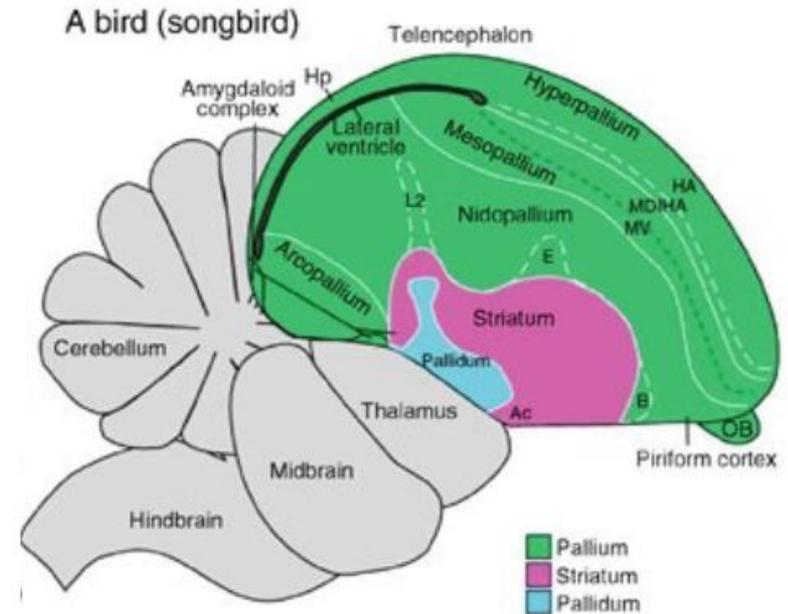
evolution → Developmental mechanisms shaping telencephalon → Huge diversity!

Elaboration of the telencephalon in amniote evolution

Birds

Hyperpallium, mesopallium & nidopallium

- Pseudo-layered structure (different from mammals)
- Apparent layers generated from VZ

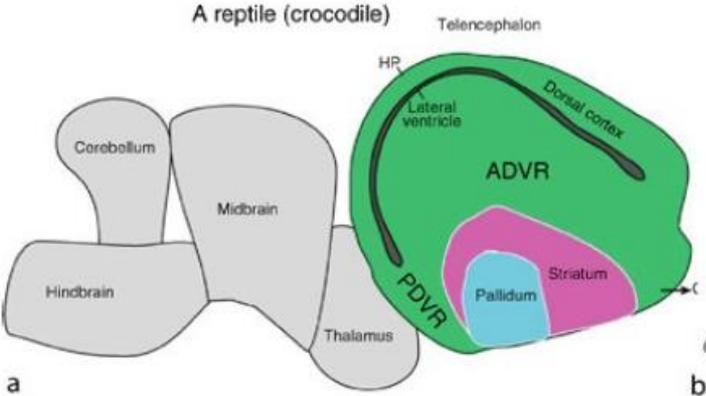


Elaboration of the telencephalon in amniote evolution

Reptiles

Dorsal cortex with 3 layers

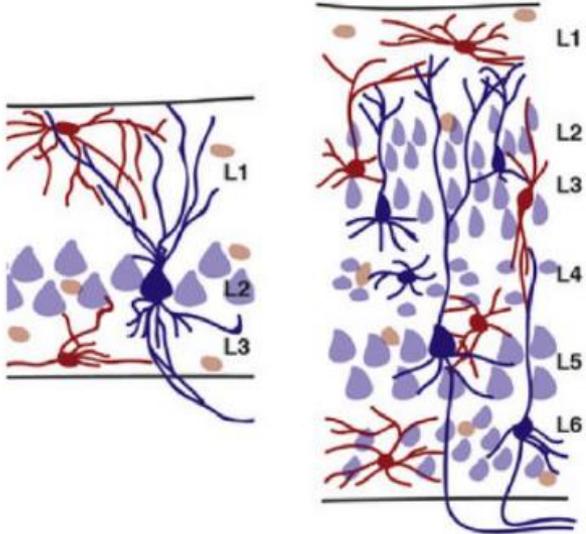
- Layers similar to mammalian layers I, V and VI
- Upper layers lack neuronal subtypes found in upper layers in mammals



three-layered dorsal cortex



six-layered neocortex



Neither birds or reptiles have homologues to the upper layer pyramidal neurons

What are the major changes in mammalian neurogenesis?

Mammalian cortex has more neurons, numerous theories for this.

Hp1
(Karten)

Mammalian neocortex evolved from rearrangement of elements already present in ancestor vertebrates.

Predicts relocation of corresponding cell groups in ancestral species.

SUPPORTING

Interneurons tangentially migrate into cortex from outside, but they only account for inhibitory, not excitatory neurons.

REJECTING

Tangential migration is not unique to mammals, also in **birds** and **reptiles**
GABAergic neurons derive from ventral telencephalon

What are the major changes in mammalian neurogenesis?

Mammalian cortex has more neurons, numerous theories for this.

Hp2
(Martinez-
Cerdeño,
Molnar)

Extracortical neurons and cortical expansion occur de novo in mammals, and it is required an accessory site of proliferation

subventricular zone

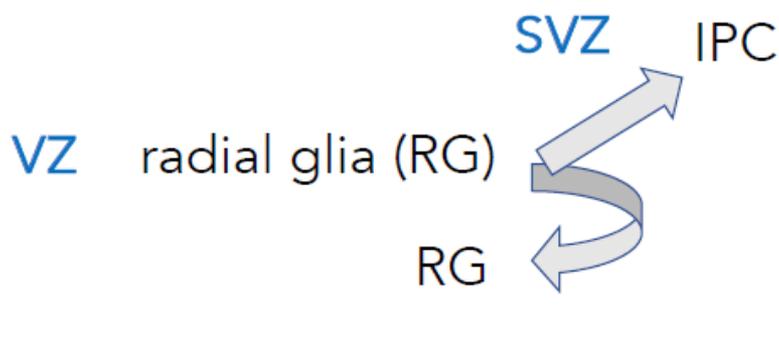
intermediate progenitors

Neurogenesis in the SVZ could increase the number of neurons produced during development

WHEN? embryonic development

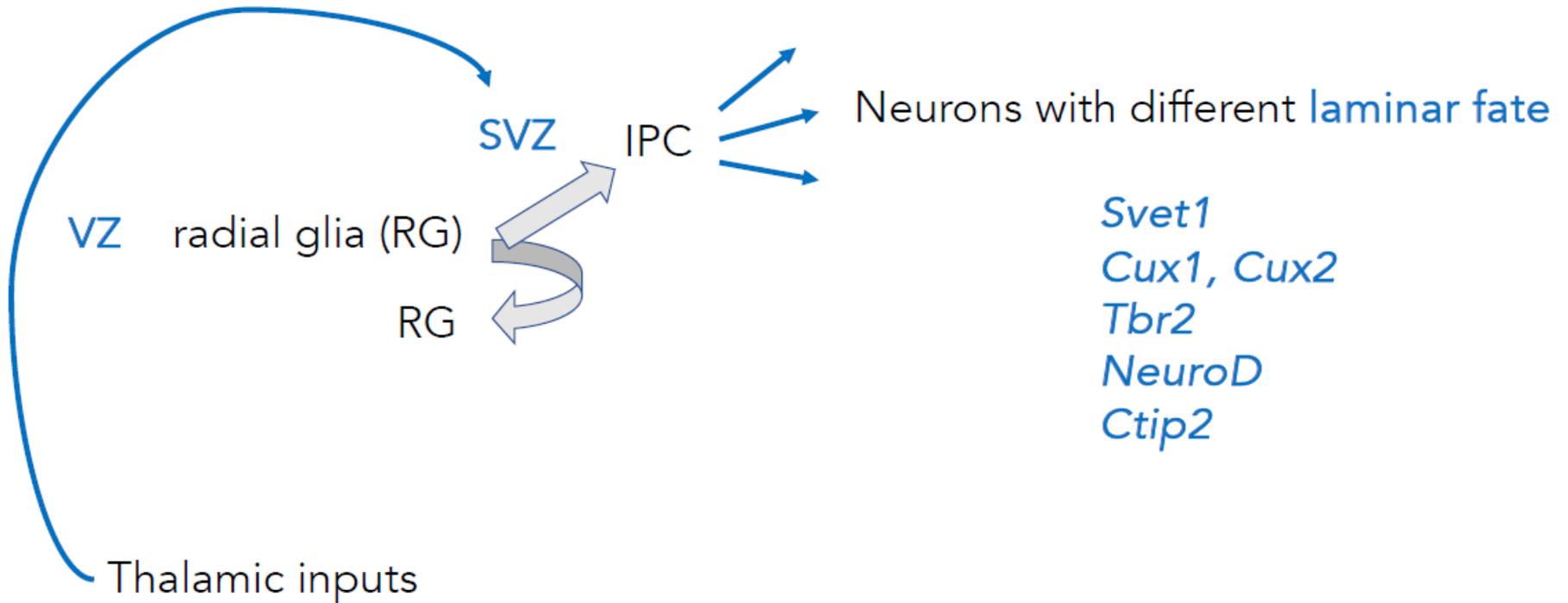
WHERE? **VZ** in all reptiles, birds and mammals

SVZ only in mammals (?)/bigger in mammals



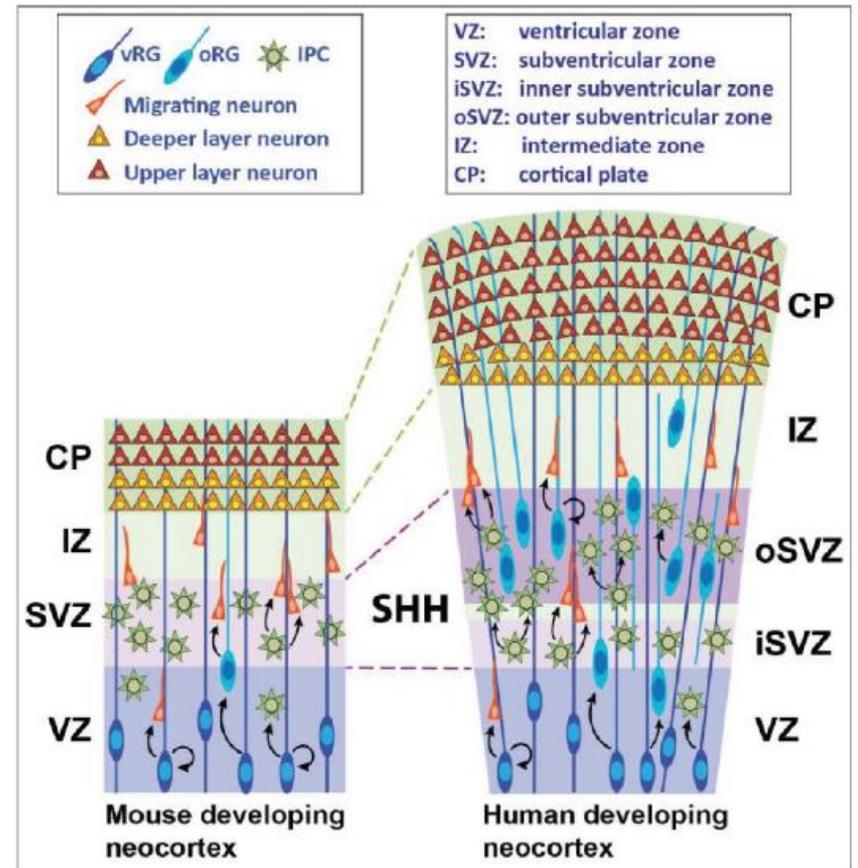
2-step neurogenesis

Neurogenesis in the SVZ could increase neural complexity



The SVZ & the expansion of the neocortex within mammals

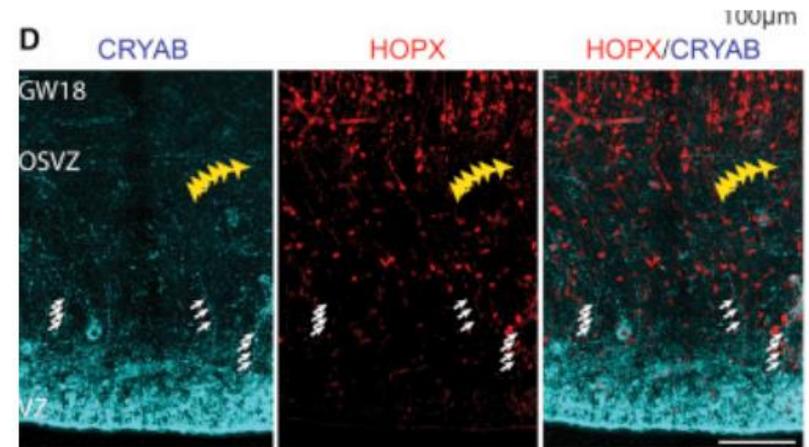
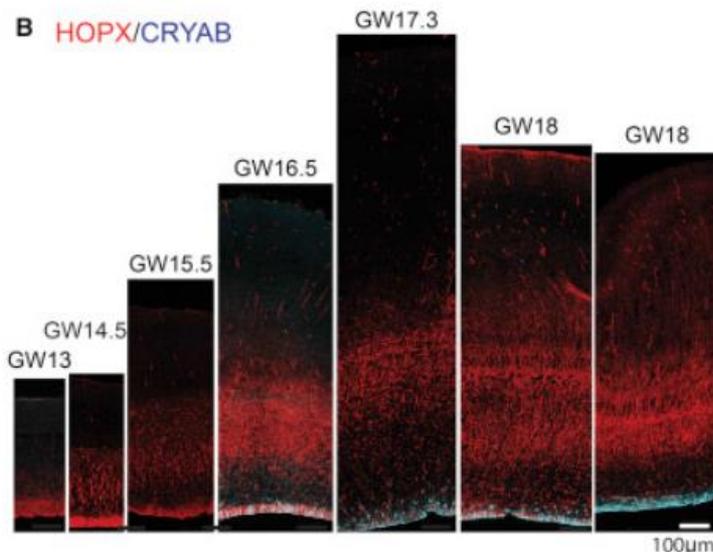
In primates, SVZ have **inner SVZ** and **outer SVZ**.



Young-Goo Han, 2016

The SVZ expansion of the neocortex within mammals

In primates, SVZ have **inner SVZ** and **outer SVZ**: different markers.

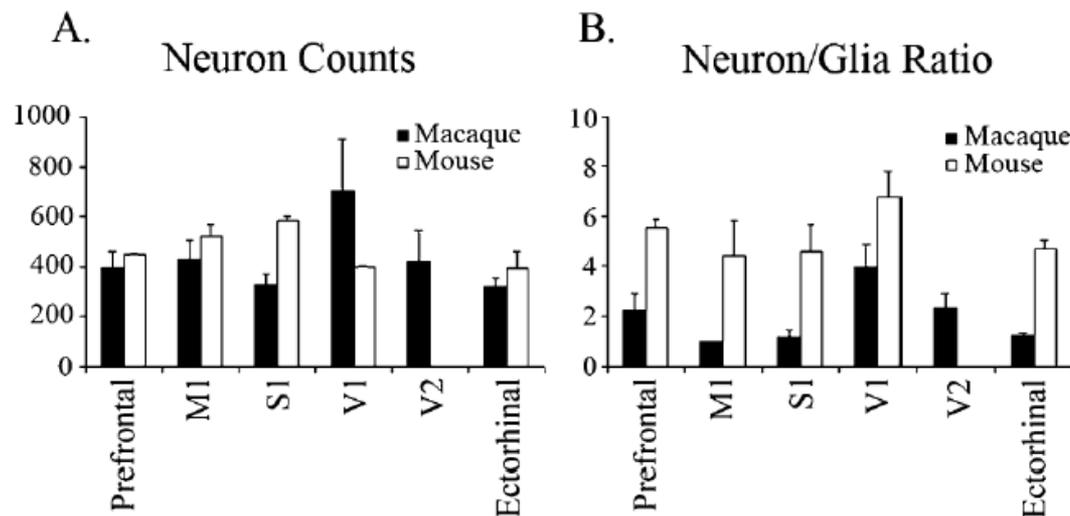


Nowakowski, 2016

Conserved neuron number in the cortical column

In mammals, cortical neurons are organized in mini-columns.

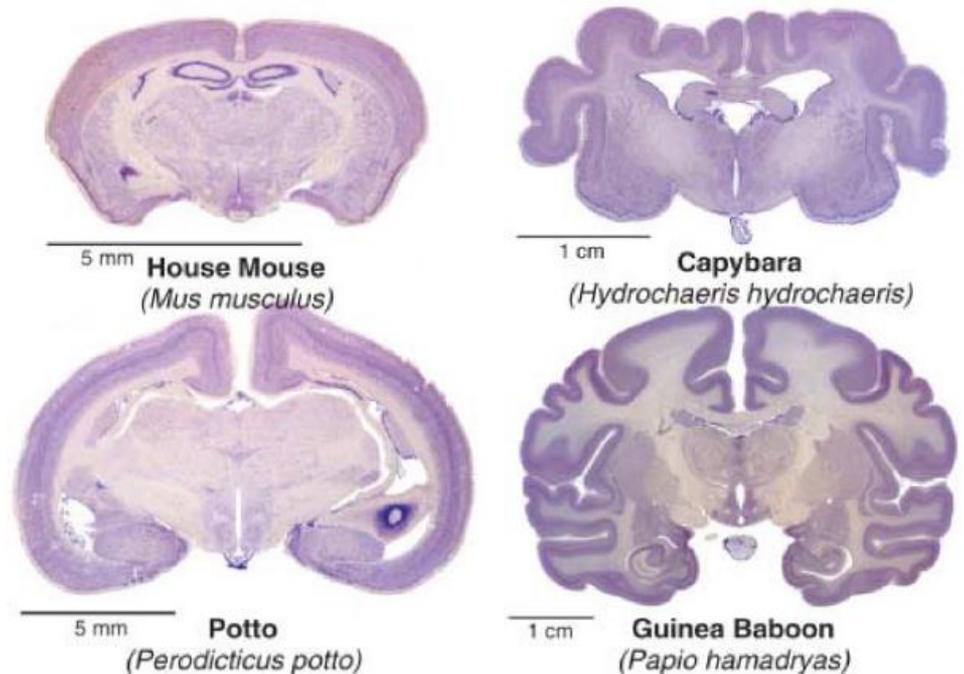
The ratio neuron/glia is decreased in primates compared to mouse, and increased in mammals compared to other species.



The SVZ and the tangential expansion of the neocortex

Transformation from lissencephalic → gyrencephalic.

Relative size of SVZ and duration of SVZ proliferation correlates with tangential expansion of the cortex.

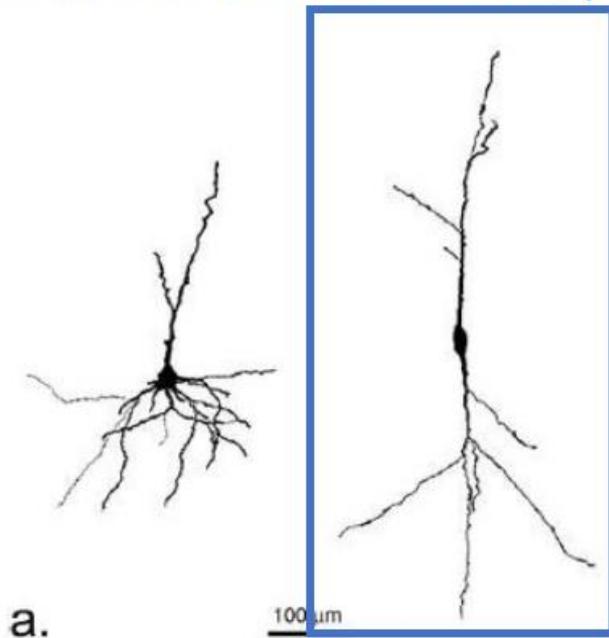


The SVZ and the neural complexity within the mammal cortex

Tangential expansion associated with unique neuronal subtypes.



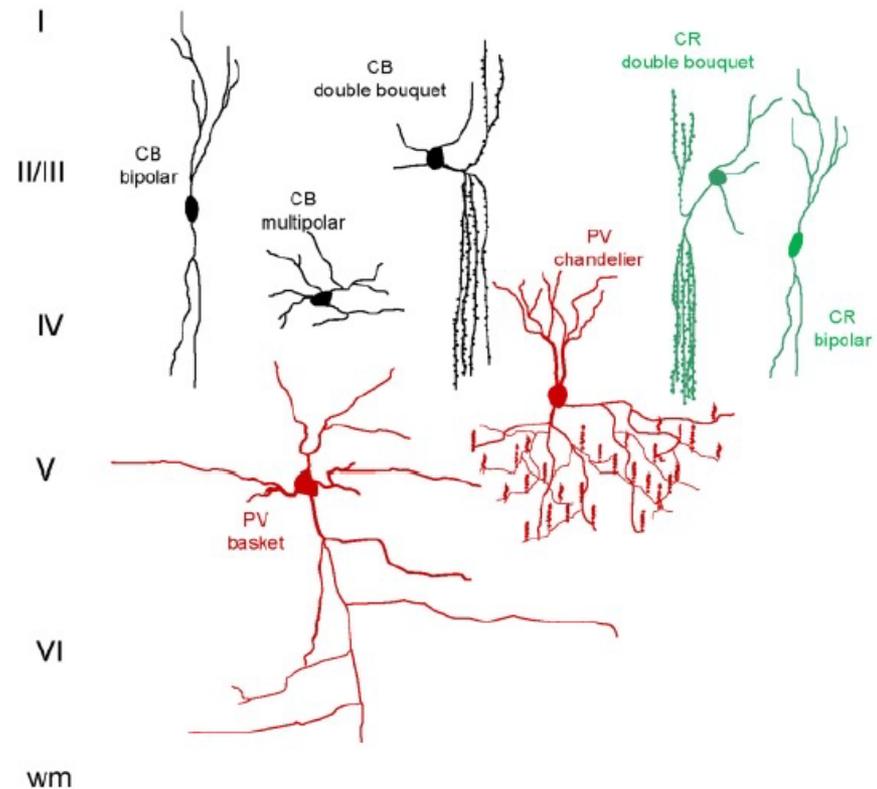
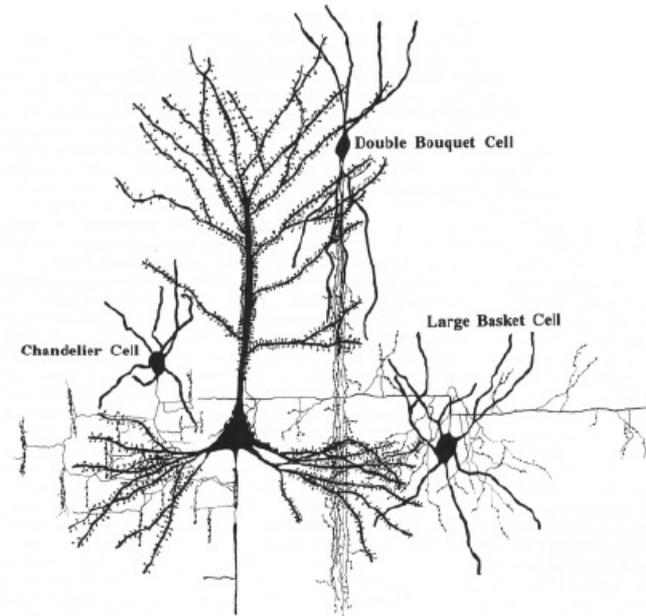
Von Economo neurons (spindle neurons)



The SVZ and the neural complexity within the mammal cortex

Tangential expansion associated with unique neuronal subtypes.

Interneuron double bouquet cells



The SVZ and the appearance of a six-layered cortex in mammals

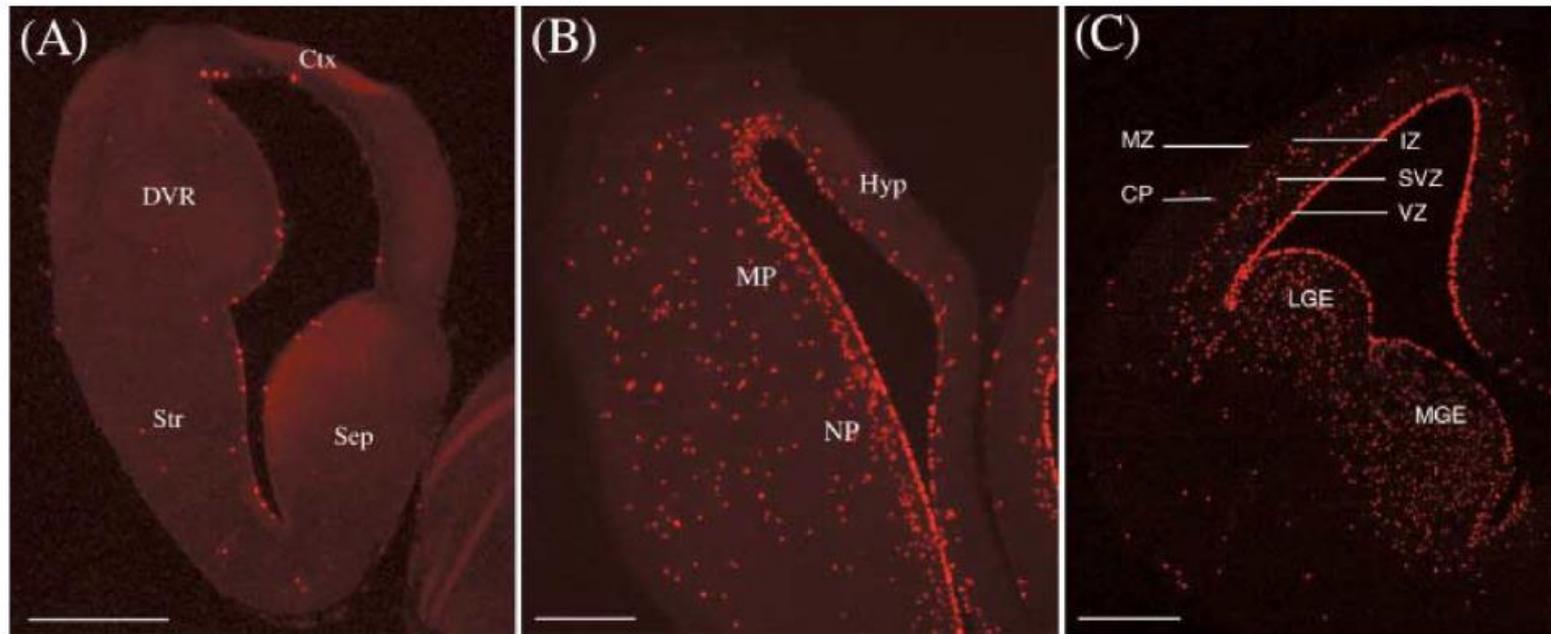
Turtles: 3-layer cortex, with neurons similar to mammalian layers I, V, and VI.

Does the turtle have SVZ?

EXP: IF anti-phospho-histone H3 in E18-E25 in turtle embryo.

The SVZ and the appearance of a six-layered cortex in mammals

pH3



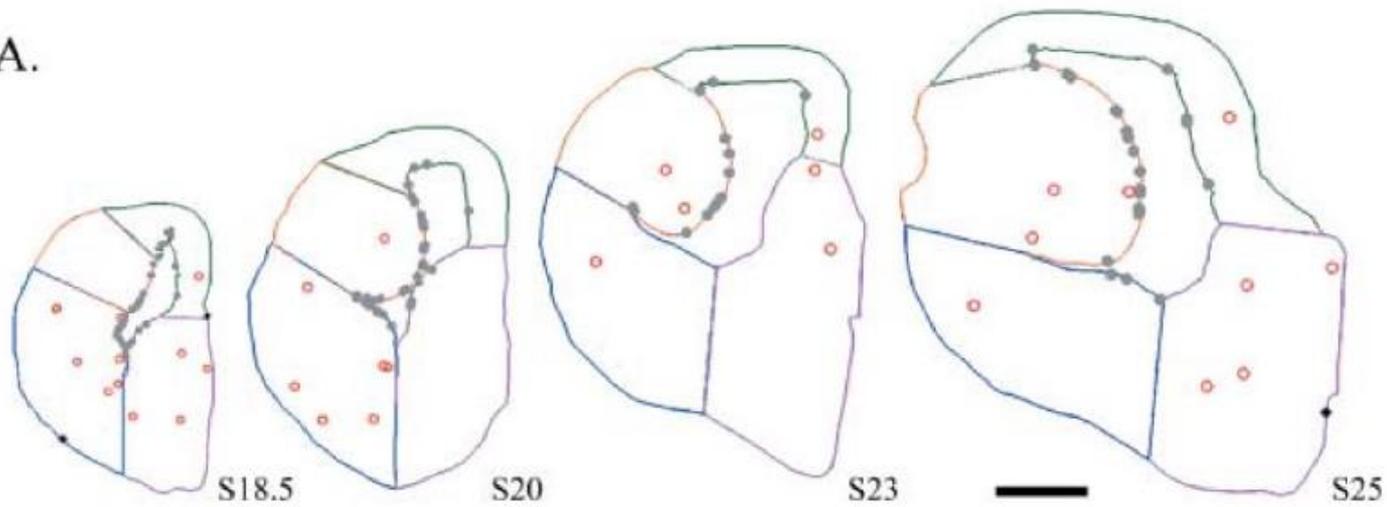
Turtle E25

Chick E8

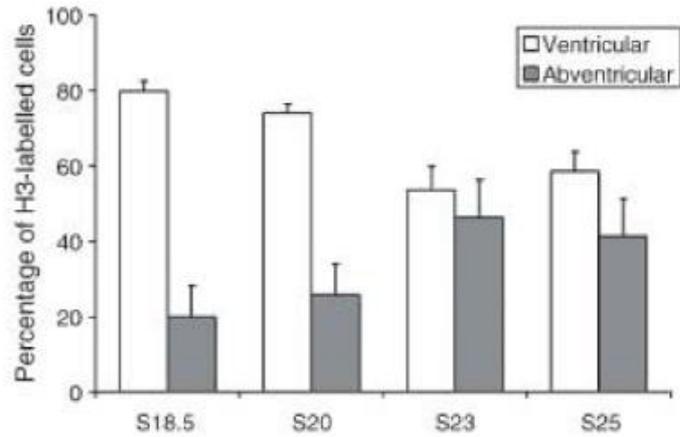
Rat E15

Turtles have a VZ, but do not have a well organized SVZ.

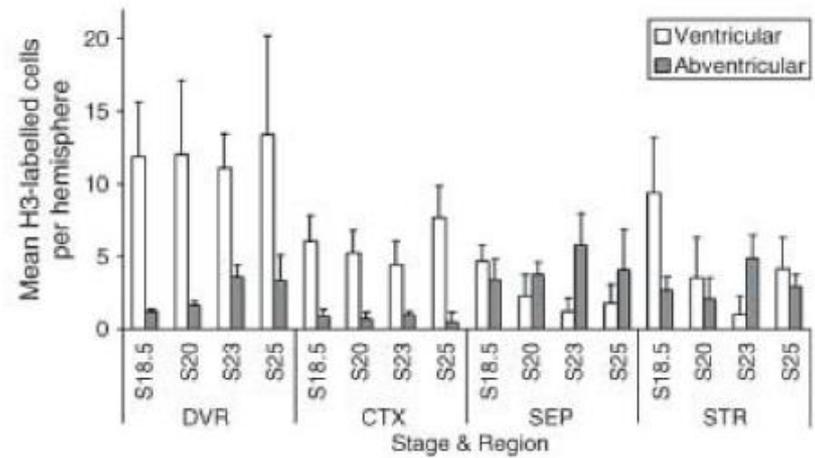
A.



B.



C.



The subcortical SVZ in mammals and birds

SVZ has been identified in subcortical regions of mammal and bird telencephalon.

Birds: Hyperpallium

Mesopallium
Nidopallium

Dorsal ventricular ridge (DVR)

Striatum & pallidum

The subcortical SVZ in mammals and birds

Birds:

SVZ absent in the hyperpallium
But present in ventral telencephalon.

Mammals:

SVZ in the LGE & MGE appears
before the pallial SVZ.

Subpallium does not form a
parallel band to the VZ.

Spatial and temporal organization of the avian SVZ

EXP: pH3 to label mitotic cells in **chicken**.

Where is the SVZ present in chicken?

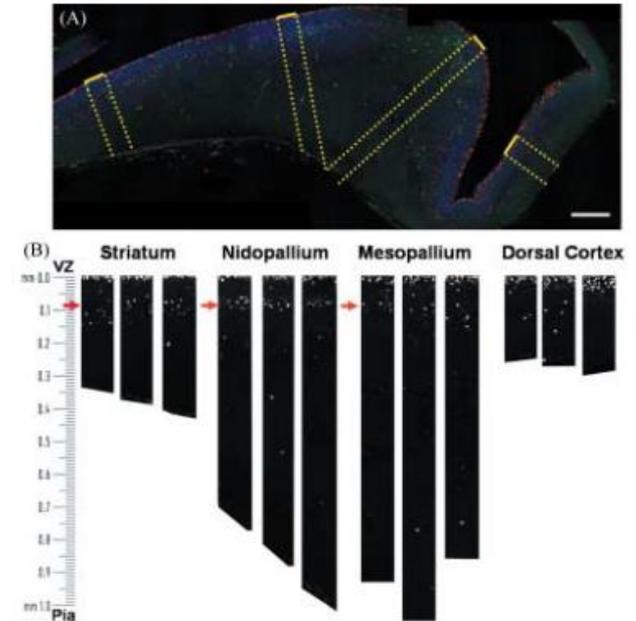


Fig. 5 Reconstructions of the H3-immunoreactive nuclei in an E8 chick telencephalon. (A) Confocal microscopic reconstruction of a coronal section of an E8 chick brain. Phospho-histone H3-labelled cells (red) were counted in 100-µm bands running perpendicular from the VZ (dotted yellow lines). Nuclei are labelled with bisbenzamide (blue) and are most dense at the VZ. The outline of the section can be observed in the green channel. Bar, 200 µm. (B) Phospho-histone H3-positive nuclei were counted in three 100-µm bands from each region. Note that H3-labelled cells (white) cluster in the VZ (within 20 µm of the ventricle) and in the SVZ starting around 90 µm from the ventricle (red arrows).

TF expression in the pallial and subpallial SVZ

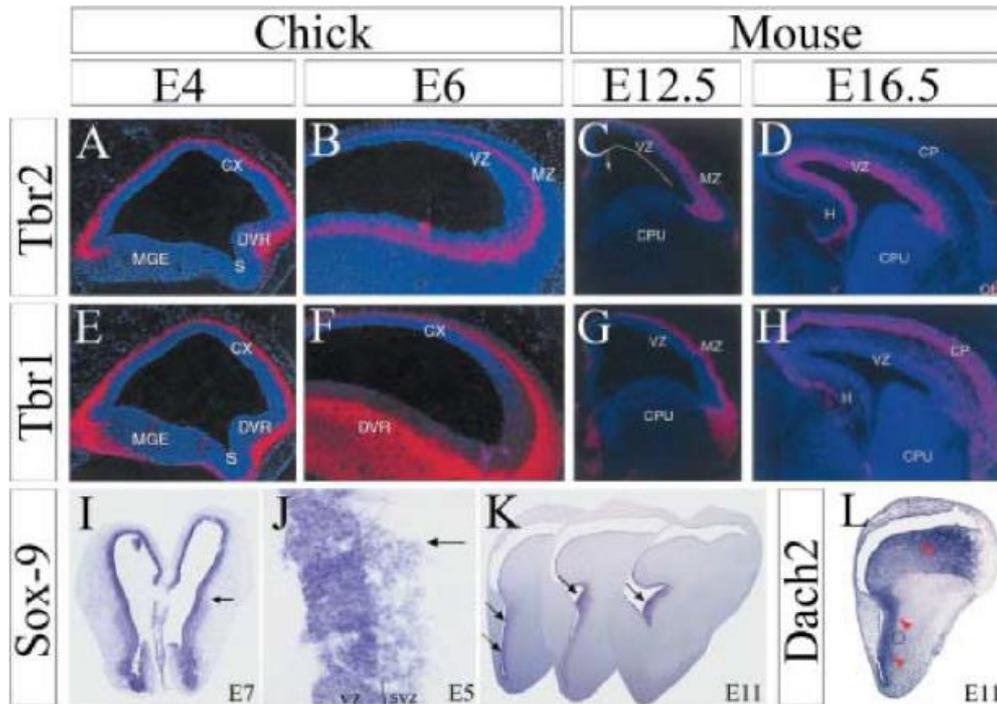
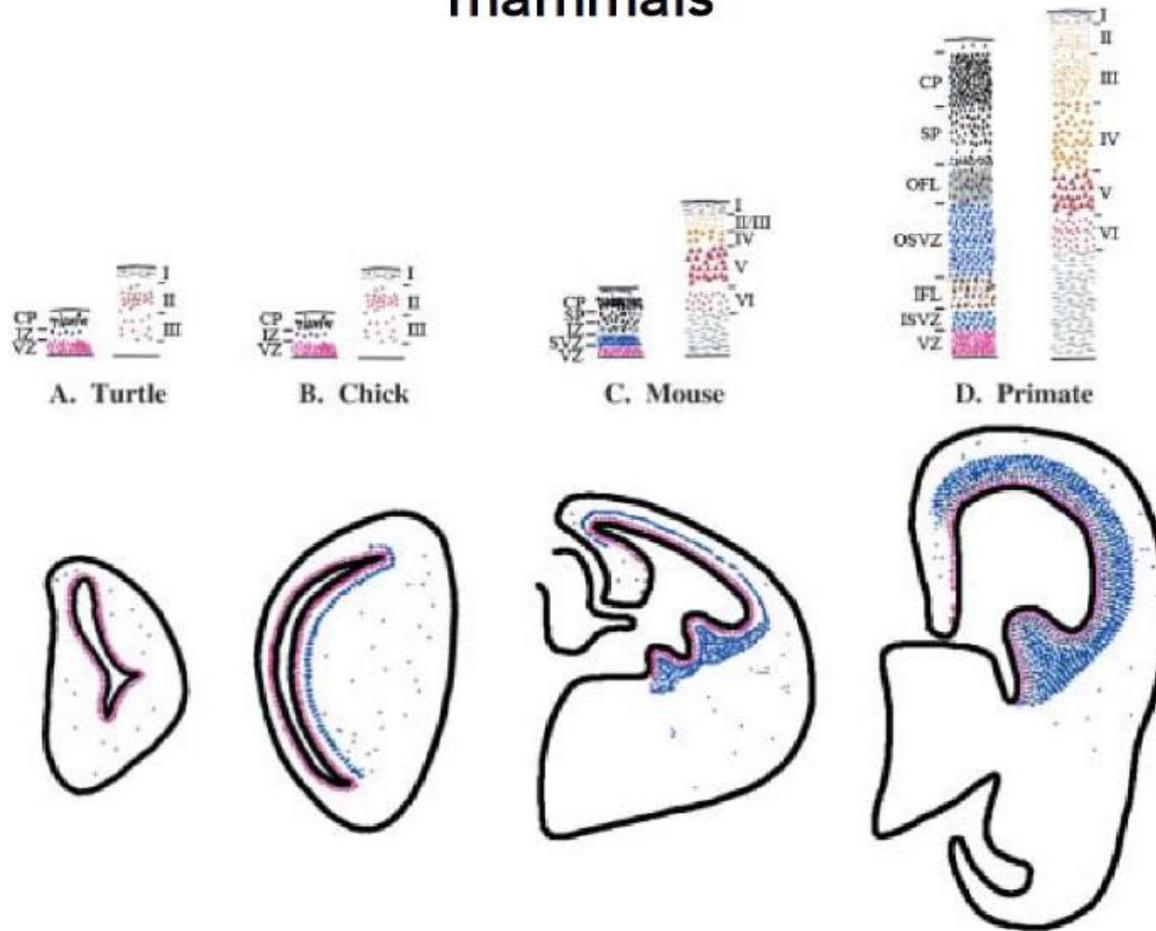


Fig. 7 Molecular markers may distinguish the pallial and subpallial SVZ in chick. *In situ* hybridization for Tbr2 in chick demonstrates that Tbr2 expression reaches the proliferative zone of the DVR at E4.5 and E6 (white arrows in C,D), and Tbr2 is excluded from the subpallium (A). In mice, Tbr2 expression reaches the cortical proliferative zone at E12.5 and E16.5 (white arrows in C,D). Tbr1 expression is confined to the periphery and restricted from the subpallium in chick (E,F) and mouse (G,H). Sox-9 is expressed all along the chick ventricular zone, but is only expressed in the subpallial SVZ at E7 (I), E5 (J) and E11 (K). Dach2 is expressed in the subpallial SVZ at E11, but also appears to express along the pallial SVZ described in this paper. Panels A–H from Bulfone et al. (1999); panels I–L from Szele et al. (2002).

Comparing sites of proliferation in chicken with reptiles and mammals



Could the pallial SVZ in birds be associated with the evolution elaboration of the avian telencephalon?

A range of adult bird species show that the size of some regions correlates with ecological and cognitive specialization.

Inside-out (radial unit) sequence of layer organization in mammals, does NOT exist in non-mammalian amniotes.

IPCs evolved maybe independently in mammals and sauropsids.



Study of pallial neurogenesis in shark embryos and the evolutionary origin of the subventricular zone

A. Docampo-Seara¹ · R. Lagadec² · S. Mazan² · M. A. Rodríguez¹ · I. Quintana-Urzainqui³ · E. Candal¹ 

Received: 9 January 2018 / Accepted: 19 June 2018 / Published online: 6 July 2018
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Introduction

Dorsal pallium in vertebrates

Absence of layers

3 layers

6 layers

Amphibians and fishes

reptiles

mammals

mammals

Neurogenesis

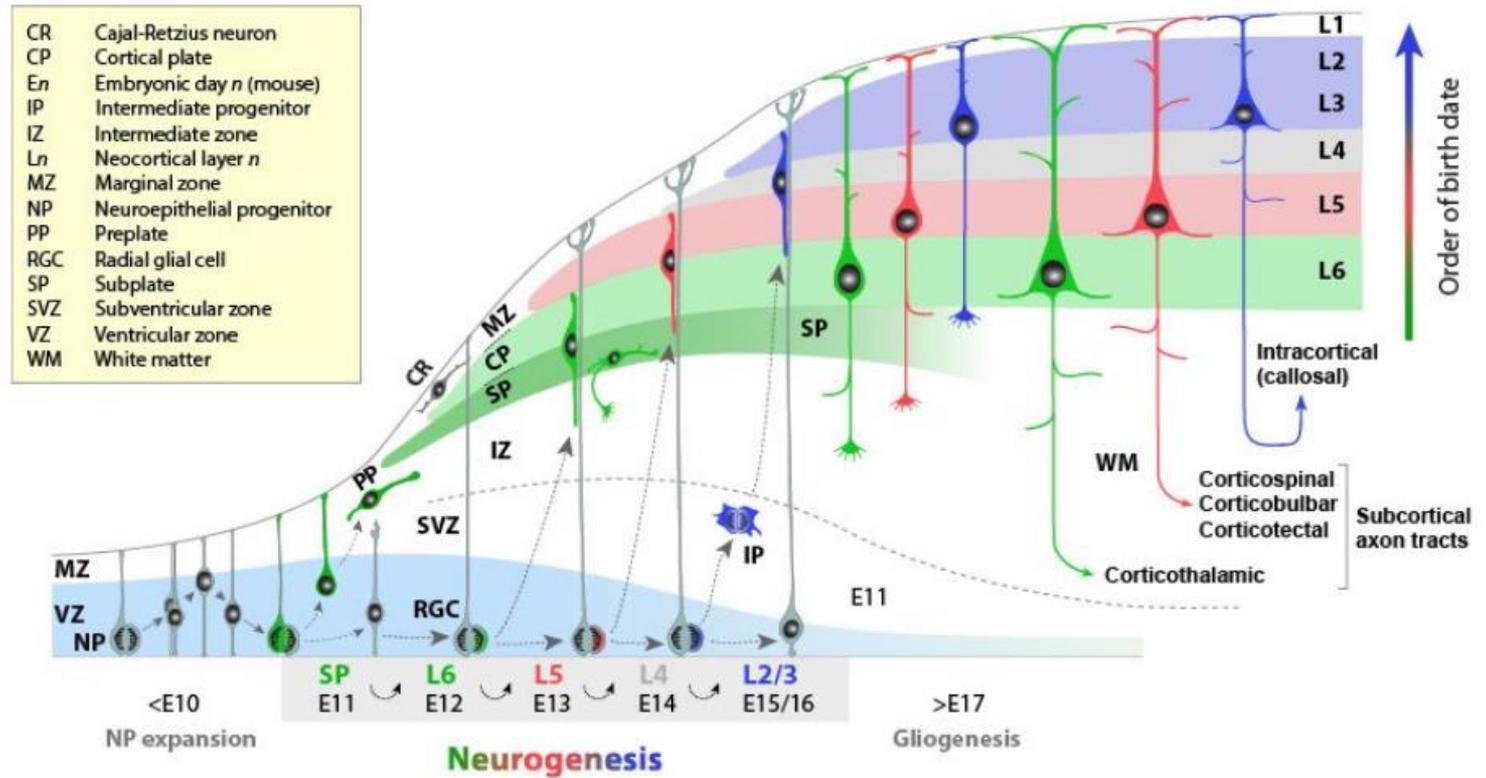


Figure 2. Neurogenesis and neuronal migration in the mouse neocortex.

mammals

Gene expression:

RG Sox2, Pax6, GFAP

IPC Tbr2

Immature neurons NeuroD

Migrating neurons Tbr1

Mature neurons Tbr1

Neurogenesis

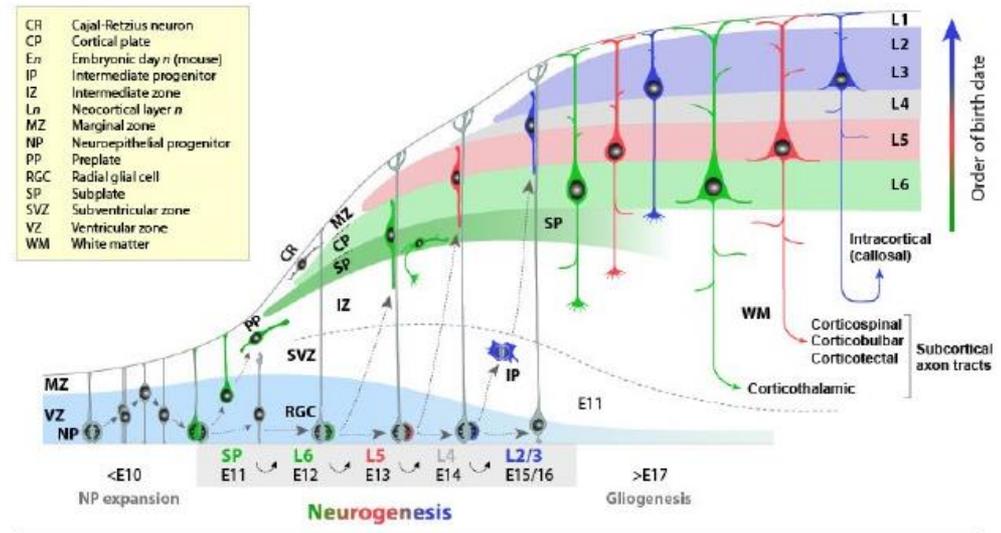


Figure 2. Neurogenesis and neuronal migration in the mouse neocortex.

Neurogenesis

RG & VZ

observed in reptiles, amphibians and teleost fishes

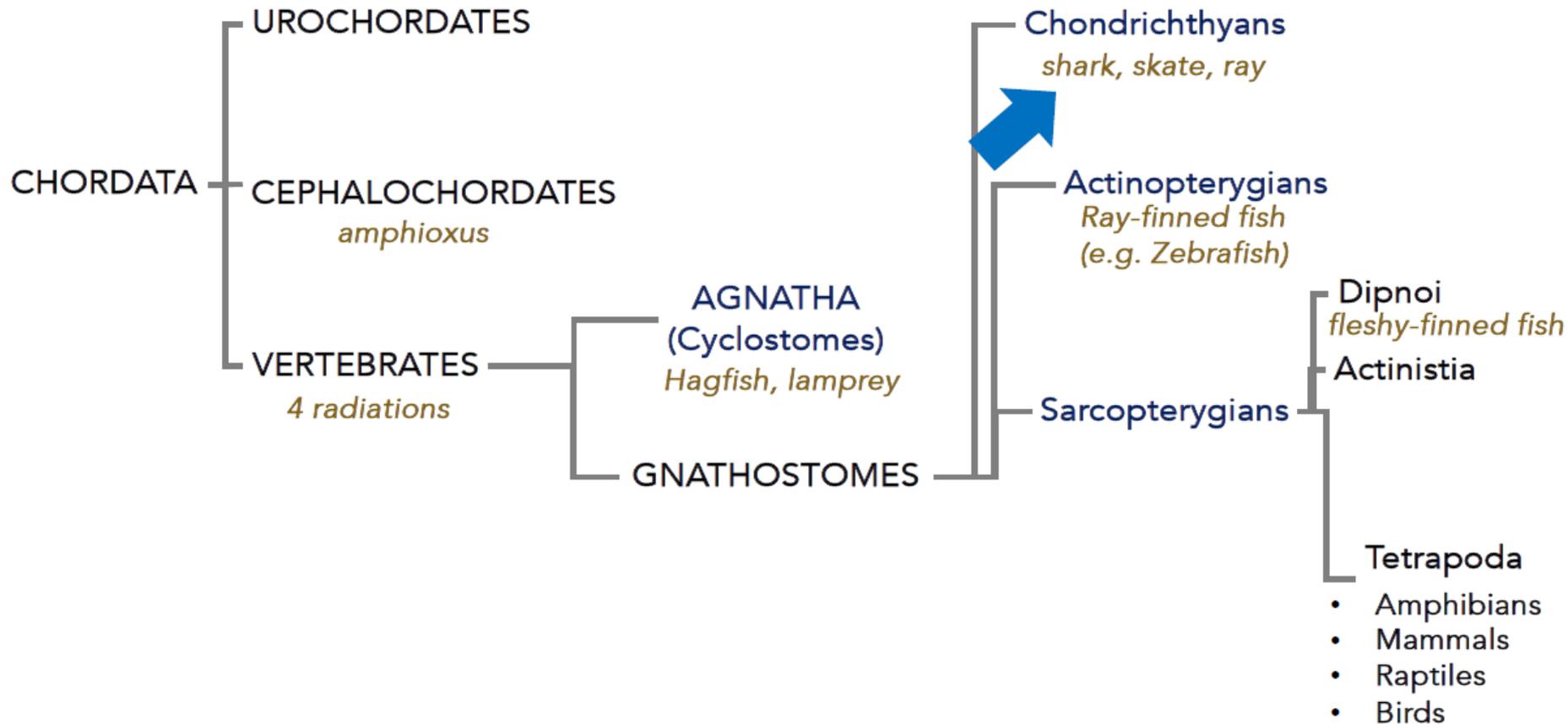
SA

Pallial SVZ

Clearly observed in some reptiles and mammals.
Here, the authors show sharks have it.

Species in this study: *Scyliorhinus canicula*

Cartilaginous fishes



AIM

Study the evolutionary mechanisms of pallial neurogenesis, the existence of IPCs and of SVZ and of glutamatergic lineage transcriptomic program in the shark.

This will tell us if some neurogenesis mechanisms were present in the common ancestor of gnathostomes.

Methods

Developmental stages: **S28-S29** early neurogenic phase

- Telencephalon subdivided in pallial domain (**Pax6**)
- & subpallial domain (**GAD65/67**)
- S28-S31 comparable to E10.5-E14.5 in mouse

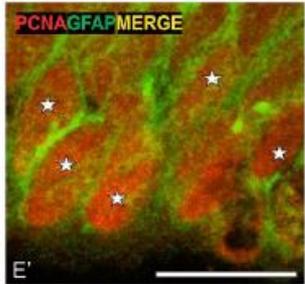
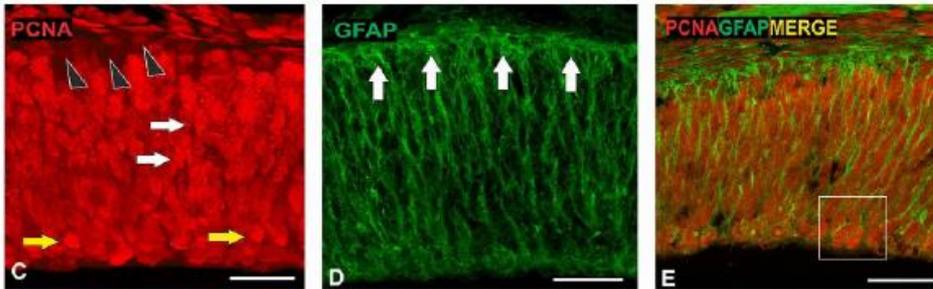
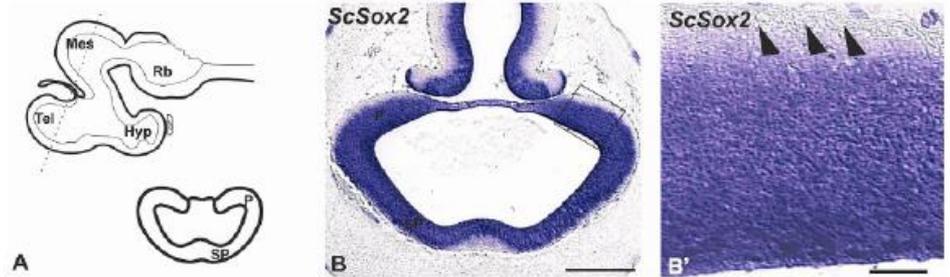
S31-S32 mid-neurogenic phase

- Telencephalon is maturing (**Pax6** still in VZ, narrower)

Immunohistological techniques

Early stages of development: characterization of pallial progenitor cells

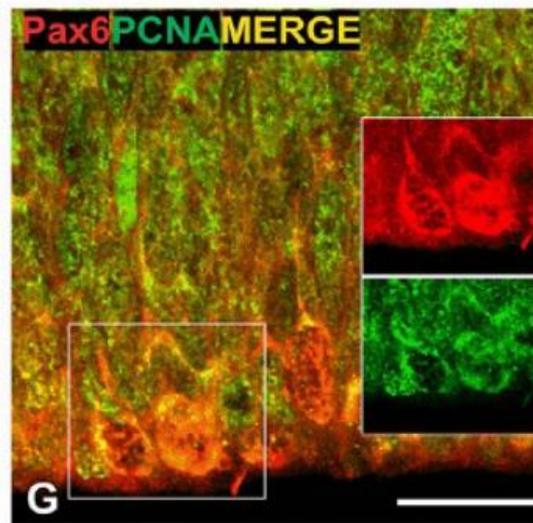
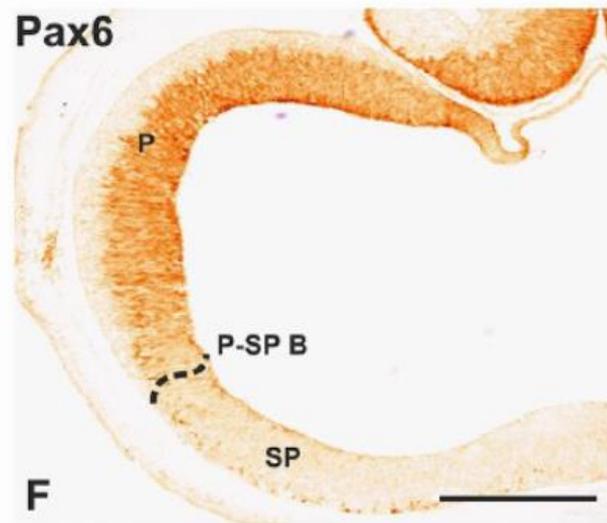
ScSox2 at S29



Do they proliferate?

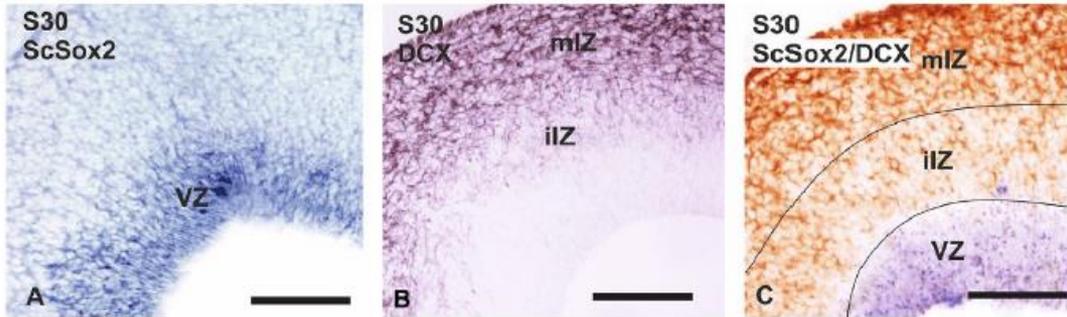
PCNA
GFAP

Early stages of development: characterization of pallial progenitor cells



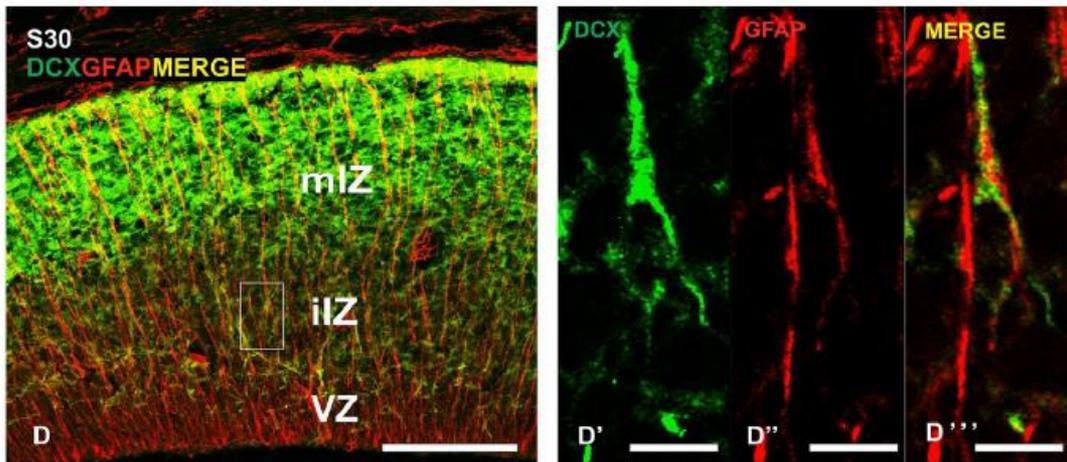
Pax6 expression

Exploring the existence of a SVZ in sharks

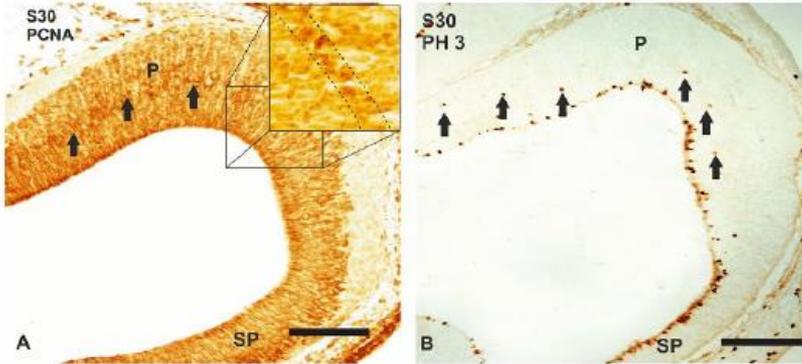


Do they proliferate and where at S30?

DCX
ScSox2

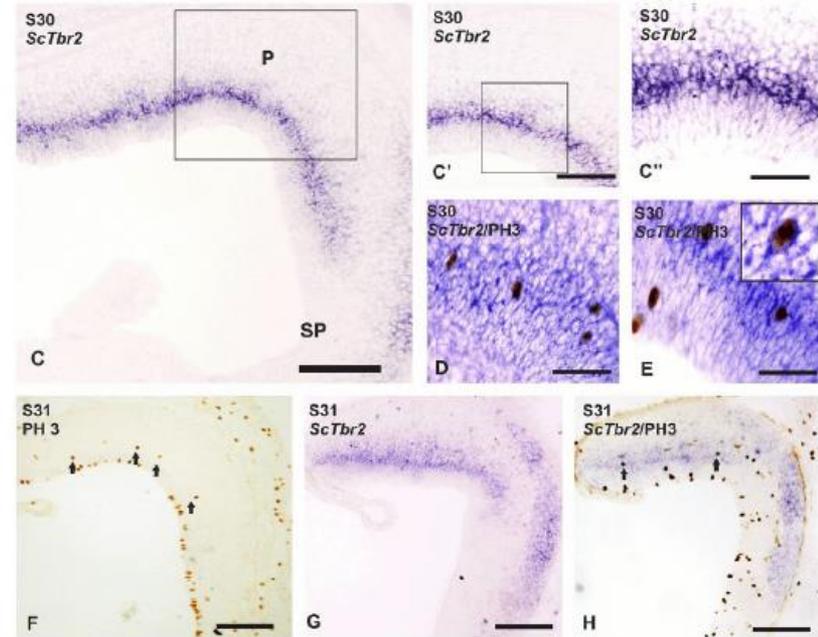


DCX
GFAP



PCNA
PH 3

ScTbr2



Exploring the existence of a SVZ in sharks

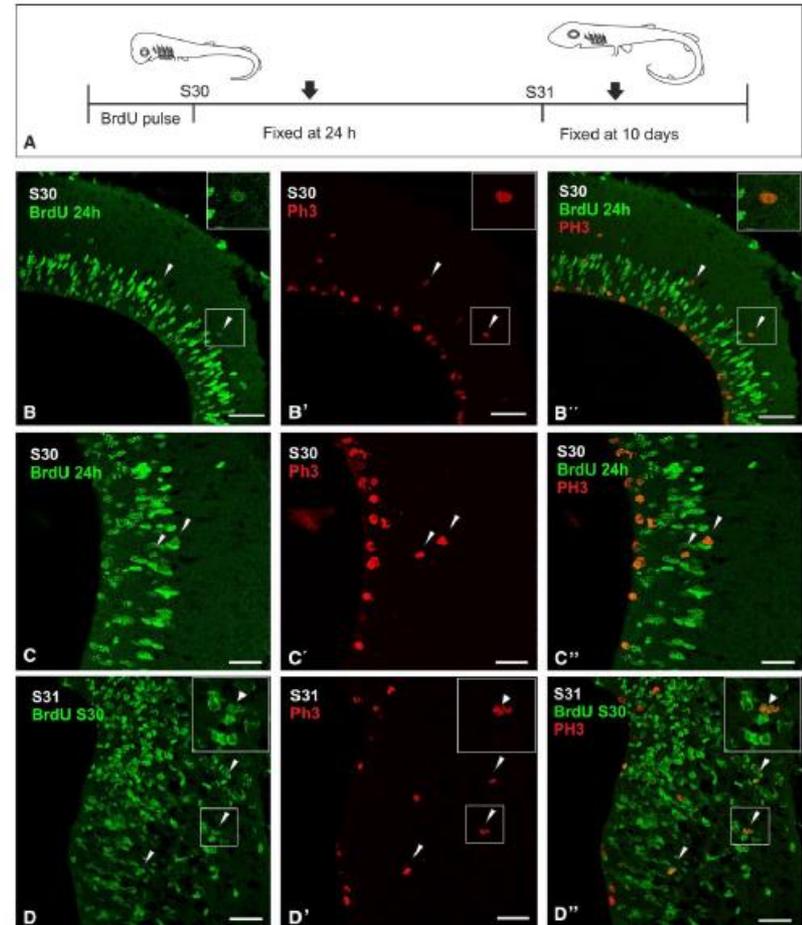
Do they proliferate and where at S30?

BrdU administration at S30 for 2.5 h



analysis after 24h or 10 days (S31)

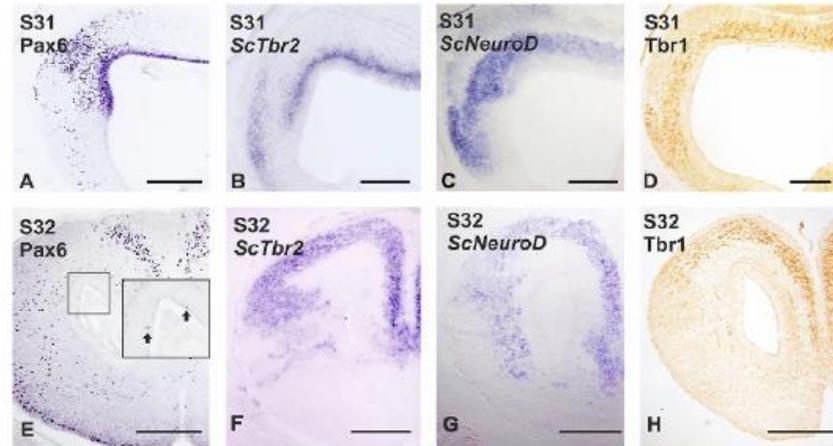
BrdU located in VZ but also in SVZ



Study of neurogenic transcription factors in S31 and S32

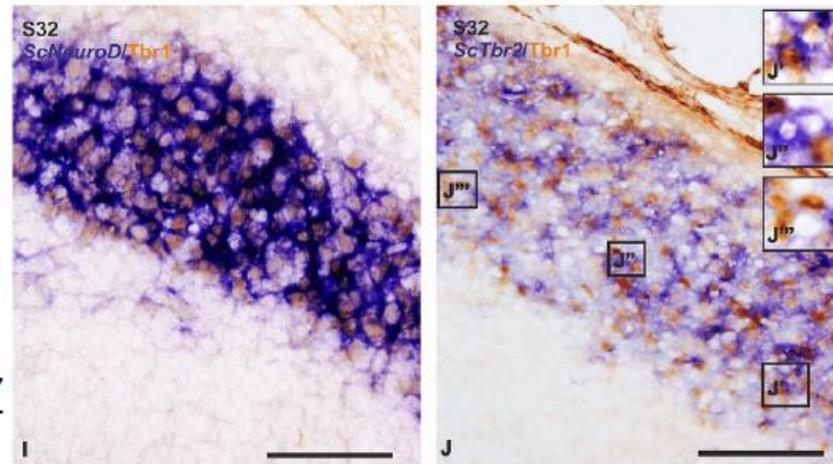
S31 mid-neurogenic phase

- *Pax6* still in VZ, narrower
- Numerous *Pax6*+ cells outside VZ
- *ScTbr2* expressed in a band above *Pax6*+ cells
- *ScNeuroD* & *Tbr2*



S32

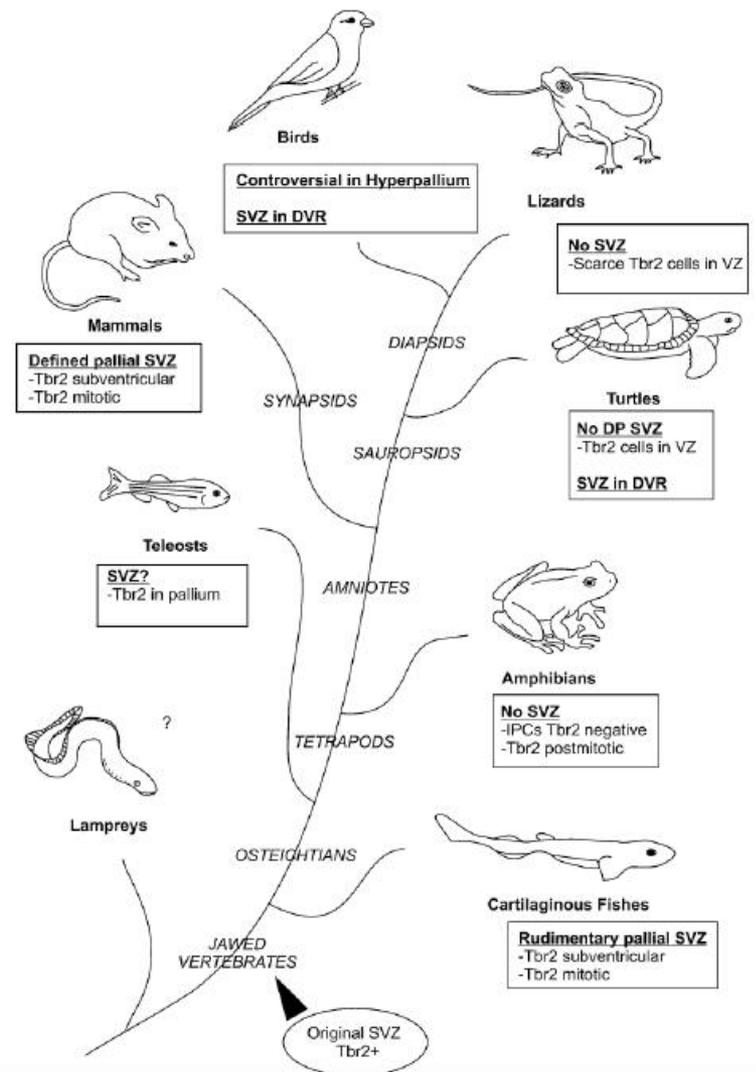
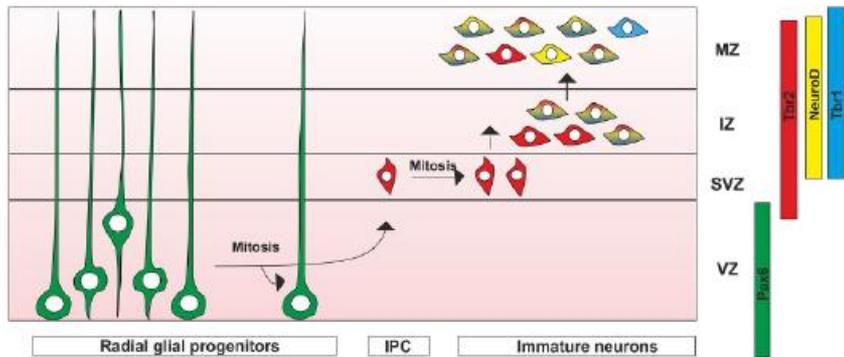
- *Pax6* not expressed anymore in the VZ
- *ScTbr2*, *ScNeuroD* & *Tbr1* not in the VZ



Discussion and Relevance

A cartilagenous fish show evidence of SVZ similar to reptiles and mammals: what is the relevance of it?

- Cartilagenous fishes are an important taxon to study early vertebrate pallium.
- Genome of cartilagenous fishes have evolved very slow.
- SVZ might be very ancient!
- Radial migration takes place in a similar way to mammals and birds



Comparative Histogenesis (II)

Neurogenesis in Zebrafish and in Xenopus

Lecture 8:

Comparative Histogenesis (II): Neurogenesis in Zebrafish and in Xenopus

1. Neurogenesis in Zebrafish

2. Neurogenesis in Xenopus

Schmidt *et al. Neural Development* 2013, **8**:3
<http://www.neuraldevelopment.com/content/8/1/3>



REVIEW

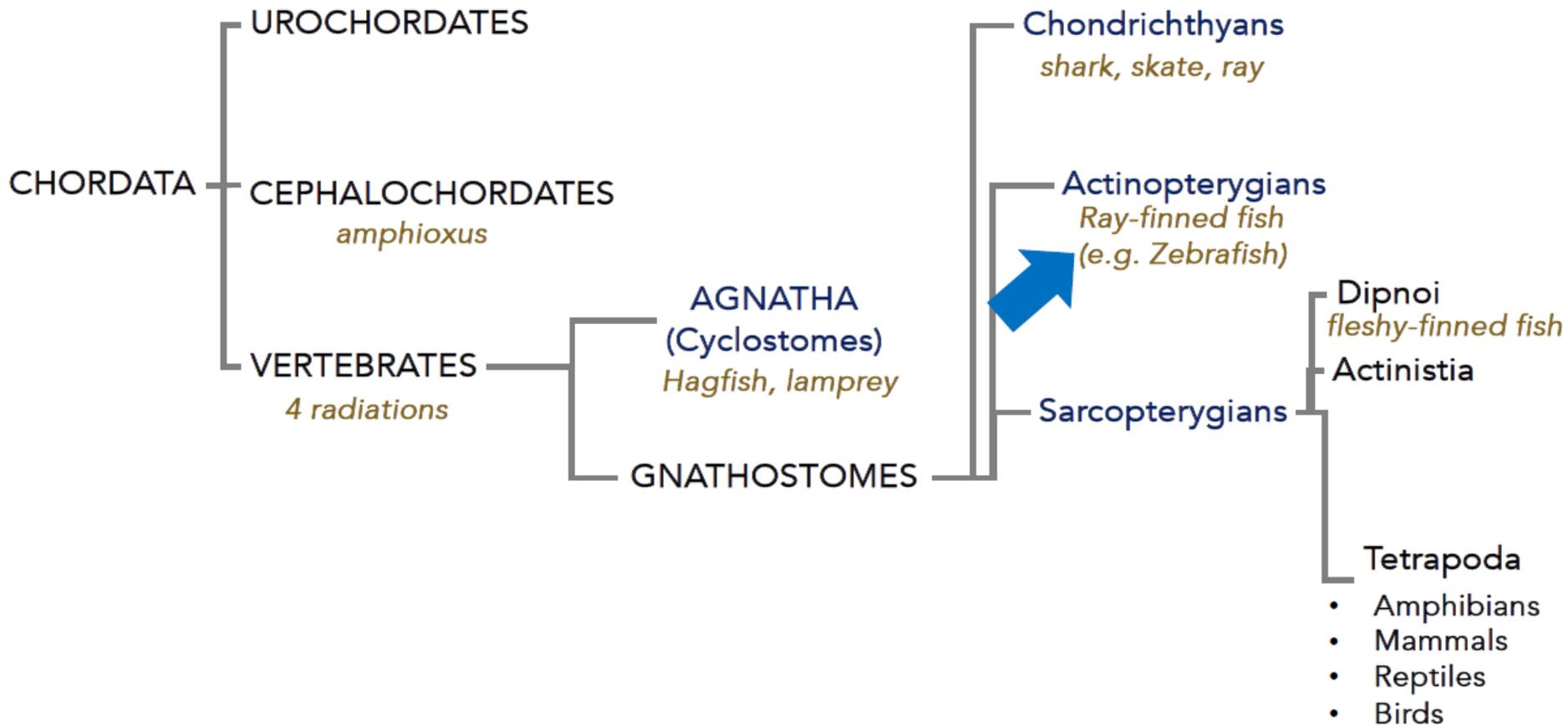
Open Access

Neurogenesis in zebrafish – from embryo to adult

Rebecca Schmidt, Uwe Strähle and Steffen Scholpp*

Species in this study: *Danio rerio*

Cartilaginous fishes



Neurogenesis in Zebrafish

- Neural induction by extrinsic factors
- Intrinsic factors
- From neural plate to neural tube
- Initiation of neurogenesis by bHLH factors, the E(Spl) family
- Early anterior-posterior patterning of the neural plate
- The thalamus neurogenesis in zebrafish

Neurogenesis in Zebrafish

- Neural induction by extrinsic factors
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Neural induction by extrinsic factors

Noggin & Chordin

Cerberus

Fgf



Neural induction



BMPs

Neurogenesis in Zebrafish

- Neural induction by extrinsic factors
- **Intrinsic factors**
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Intrinsic factors

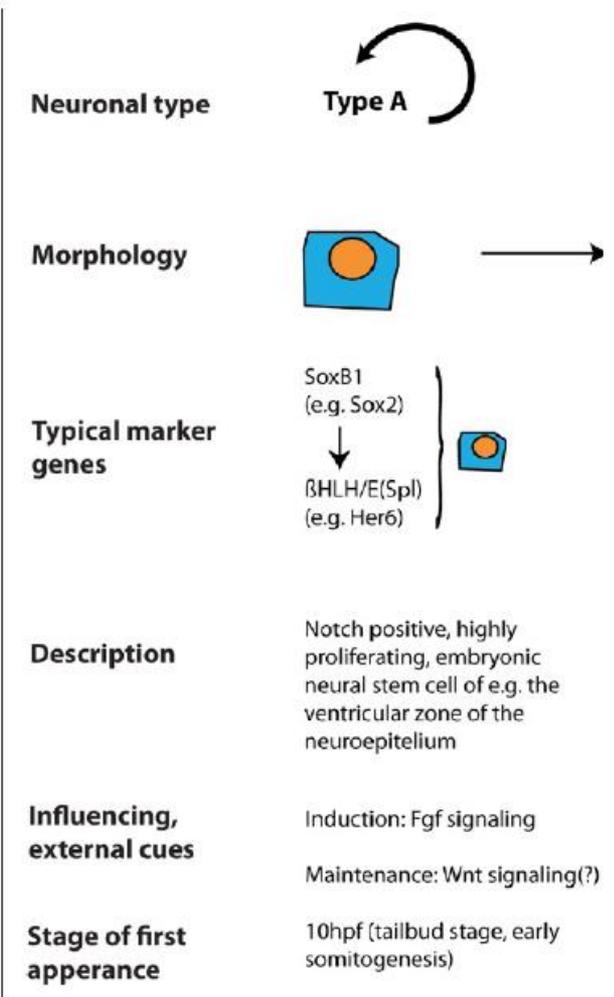
SoxB1 (e.g. Sox2)

Specification of embryonic ectoderm into type A neuroectodermal lineage (in vertebrates)

In zebrafish:

Sox1 (a/b), 2, 3, 19(a/b) Blastula stage

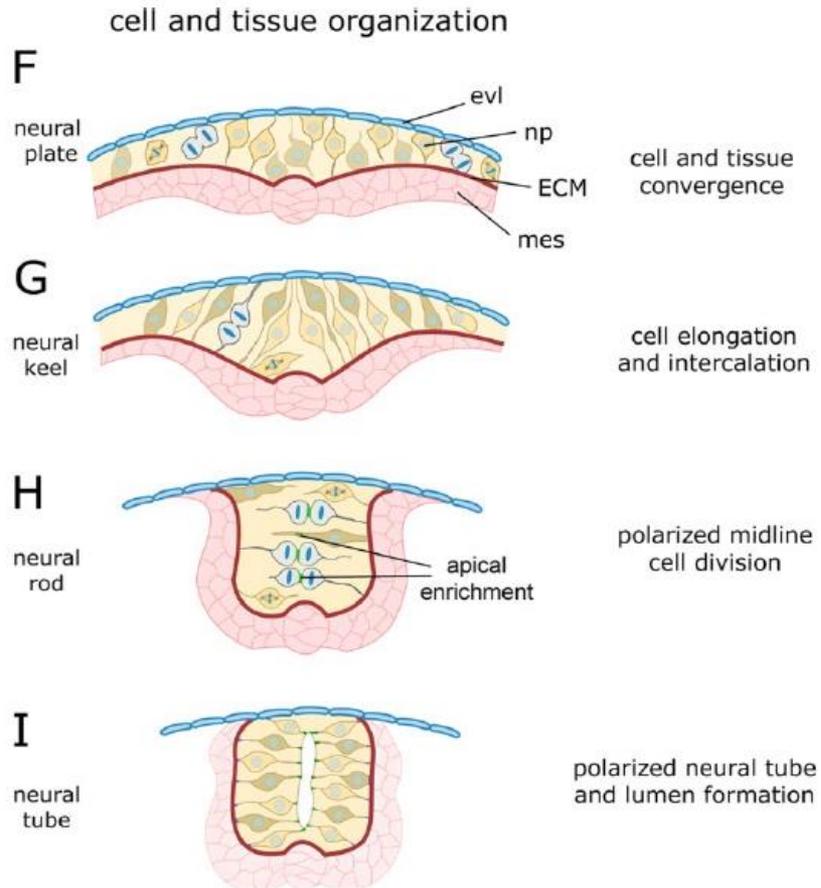
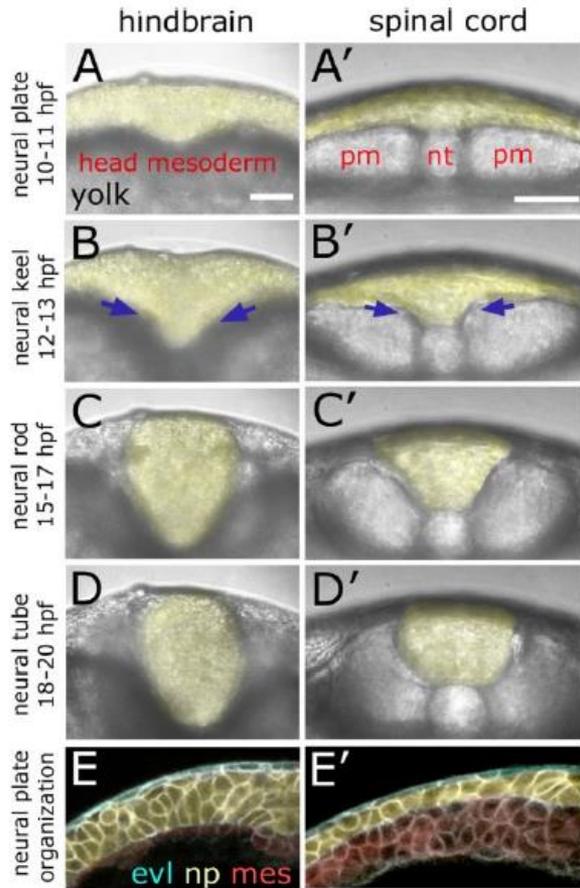
Sox2 NSCs



Neurogenesis in Zebrafish

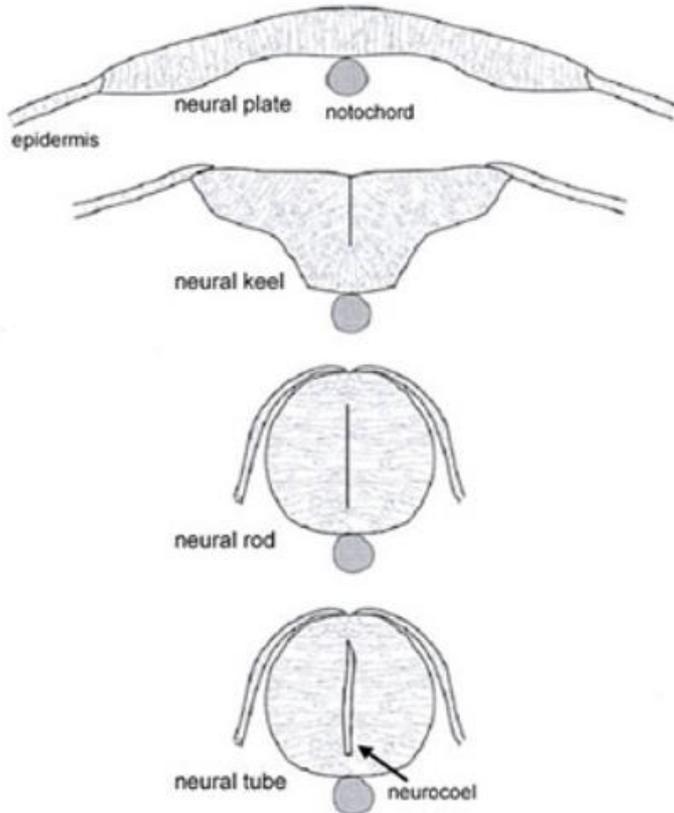
- Neural induction by extrinsic factors
- Intrinsic factors
- **From neural plate to neural tube**
- Initiation of neurogenesis by bHLH factors, the E(Spl) family
- Early anterior-posterior patterning of the neural plate
- The thalamus neurogenesis in zebrafish

From neural plate to neural tube



Araya et al., 2015

From neural plate to neural tube



Basal self-renewal regulated by Notch

Interkinetic nuclear migration

Neurogenesis in Zebrafish

- Neural induction by extrinsic factors
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- The thalamus neurogenesis in zebrafish

Neurogenesis in Zebrafish

- Neural induction by extrinsic factors
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- **Early anterior-posterior patterning of the neural plate**
- The thalamus neurogenesis in zebrafish

Early anterior-posterior patterning of the neural tube

Wnt

Fgf

Nodal

Retinoic acid

in neural plate primordium

in Zebrafish

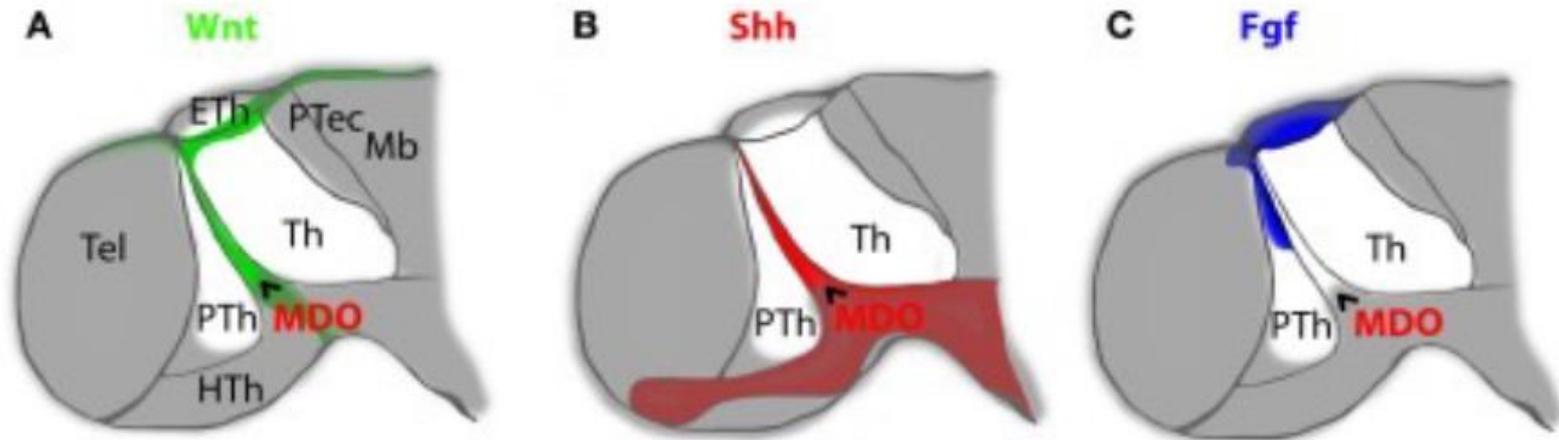
Tgf3 & axin → anterior patterning and eye

ANB anterior neural boundary

- organizer to pattern the anterior neural plate
- releases SFRP —| Wnt

Early anterior-posterior patterning of the neural tube

MDO mid-diencephalic organizer → **Shh**



MHB isthmus rhombencephali organizer → **Fgf8**

Neurogenesis in Zebrafish

- Neural induction by extrinsic factors
- Intrinsic factors
- From neural plate to neural tube
- Initiation of neurogenesis by bHLH factors, the E(Spl) family
- Early anterior-posterior patterning of the neural plate
- **The thalamus neurogenesis in zebrafish**

The thalamus neurogenesis in zebrafish

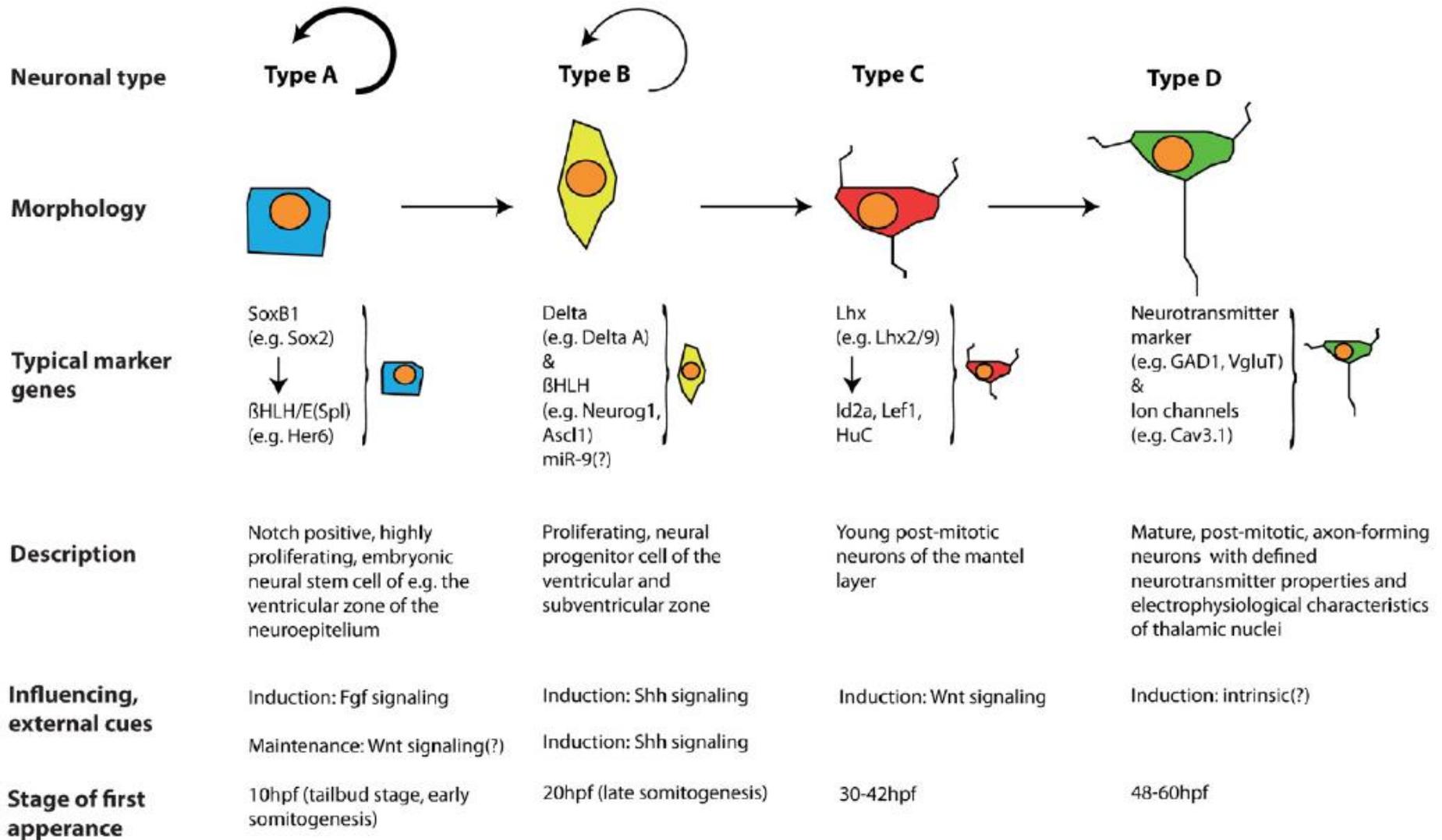
In the thalamus, neuronal differentiation progresses from posterior to anterior (neurogenetic gradient).

MDO → Shh → neurog1 → neurogenesis

E(Spl)

Her6+ domains remain separated and will undergo delayed neurogenesis

Neurogenesis in the embryonic thalamus in zebrafish





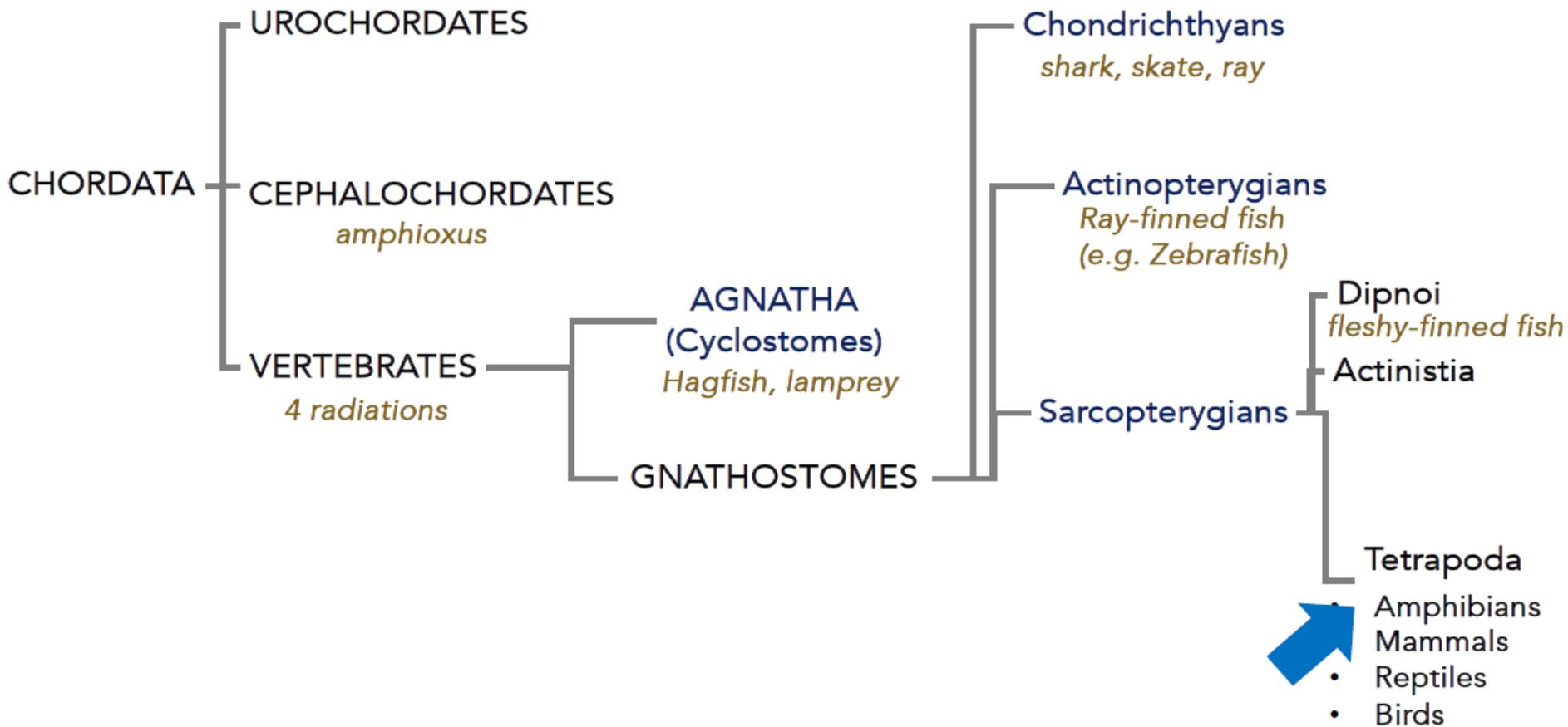
Pattern of Neurogenesis and Identification of Neuronal Progenitor Subtypes during Pallial Development in *Xenopus laevis*

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Species in this study: *Xenopus laevis*

Cartilaginous fishes



Xenopus embryonic and larval development

TABLE 1 | *Xenopus laevis* developmental timing at 23°C.

| | | | | | | | |
|--|---|---|--|--|--|--|---|
|  |  |  |  |  |  |  |  |
| Stage 28 1 day, 8 hr 30 min | Stage 31 1 day, 13 hr 30 min pf | Stage 35–36 2 days, 2 hr pf | Stage 37–38 2 days, 5 hr 30 min pf | Stage 40 2 days, 18 hr pf | Stage 42 3 days, 8 hr pf | Stage 46 4 days, 10 hr pf | Stage 54 26 days pf |
| Early embryonic stages | | | Late embryonic stages | | | Larval stages | |
| Neural tube appearance | | | Before telencephalic evagination | Incipient telencephalic evagination | Telencephalon evaginated | | |
|  | | |  |  |  |  | |

Xenopus larval development



Pre-metamorphosis
(St 46 - 52)

Pro-metamorphosis
(St 43 - 58)

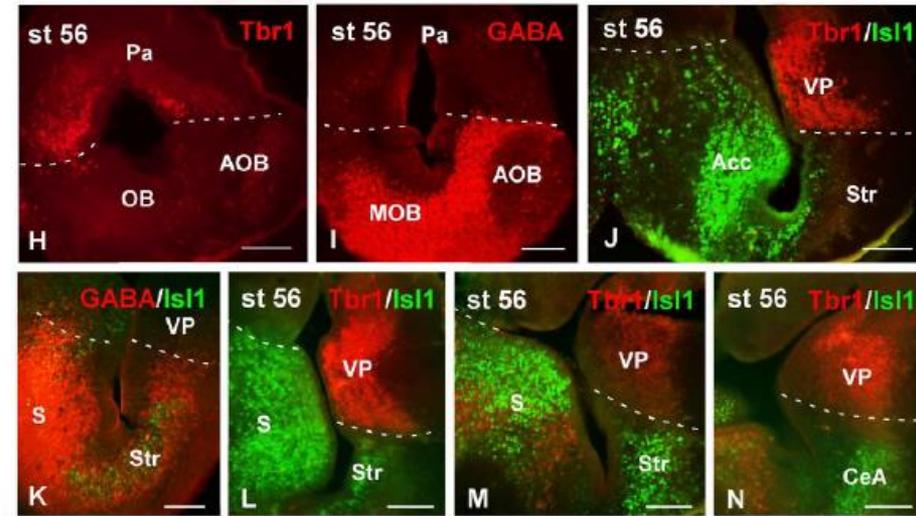
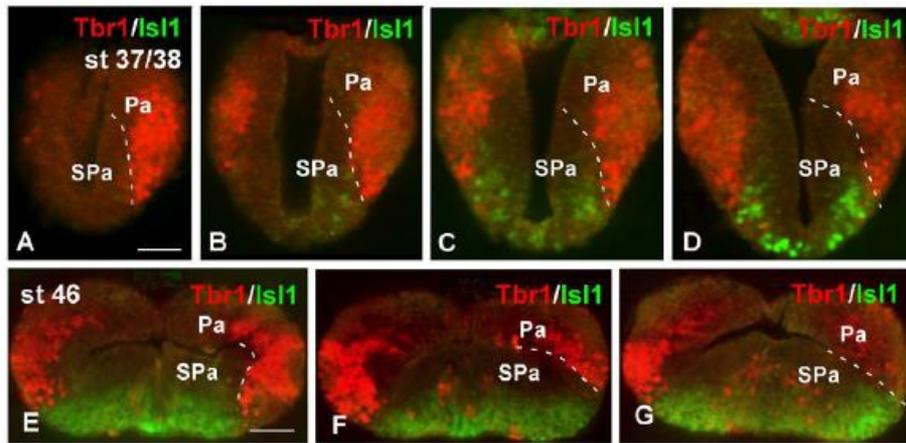
Metamorphic climax
(St 59 - 65)

Metamorphosized juvenile
(St 65 - 66)

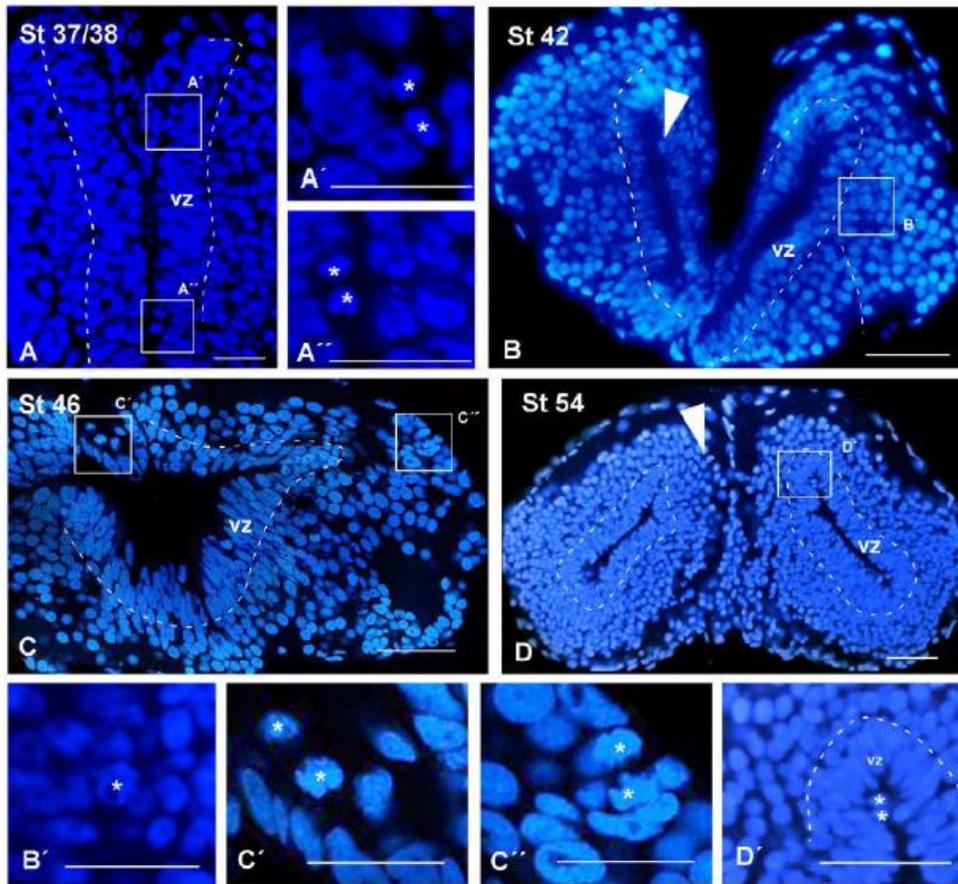
Pallial boundaries during development

Tbr1: marker of pallium

Isl1: marker of subpallium

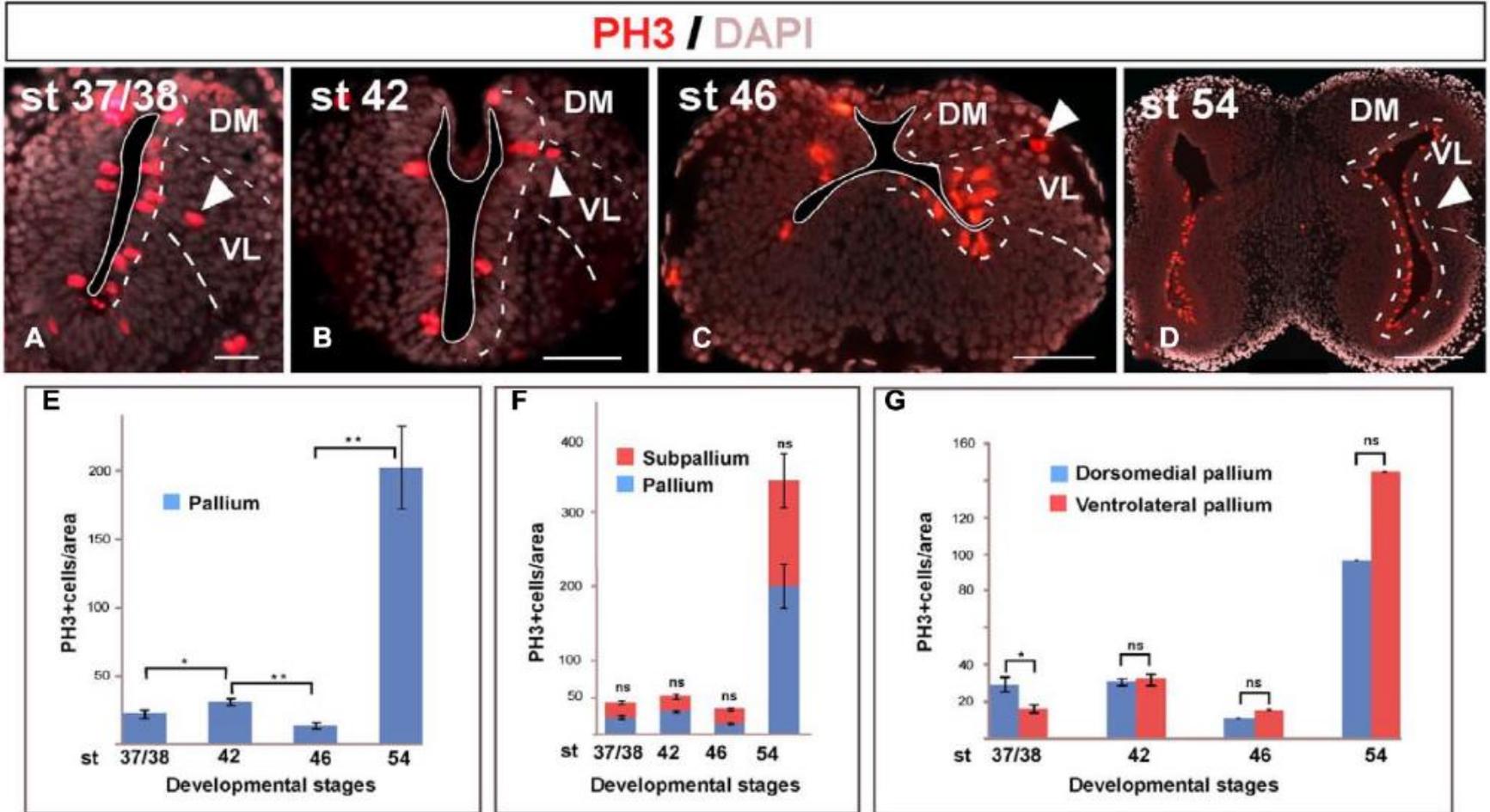


Ventricular limits and mitotic cells

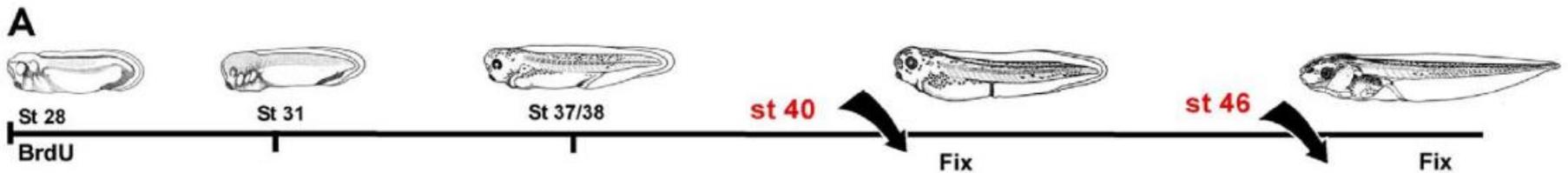


DAPI: to localize chromatine content
& localize mitotic cells

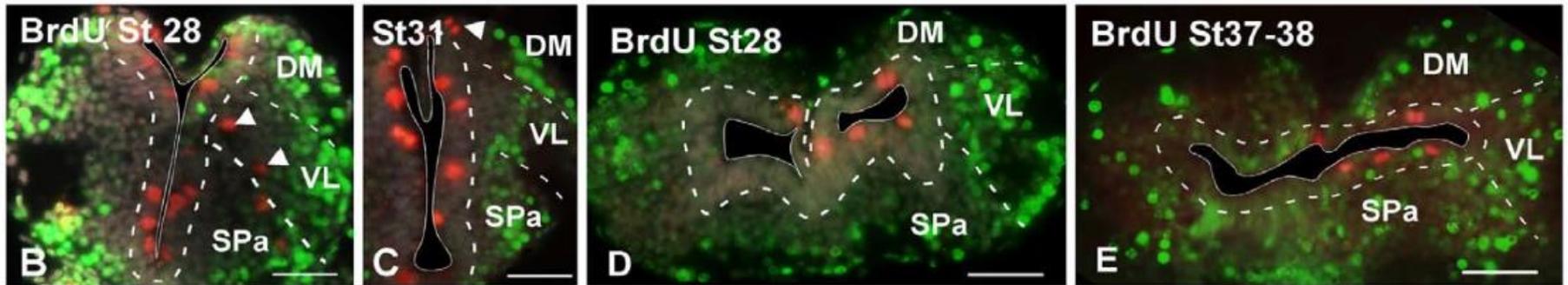
Mitotic rate



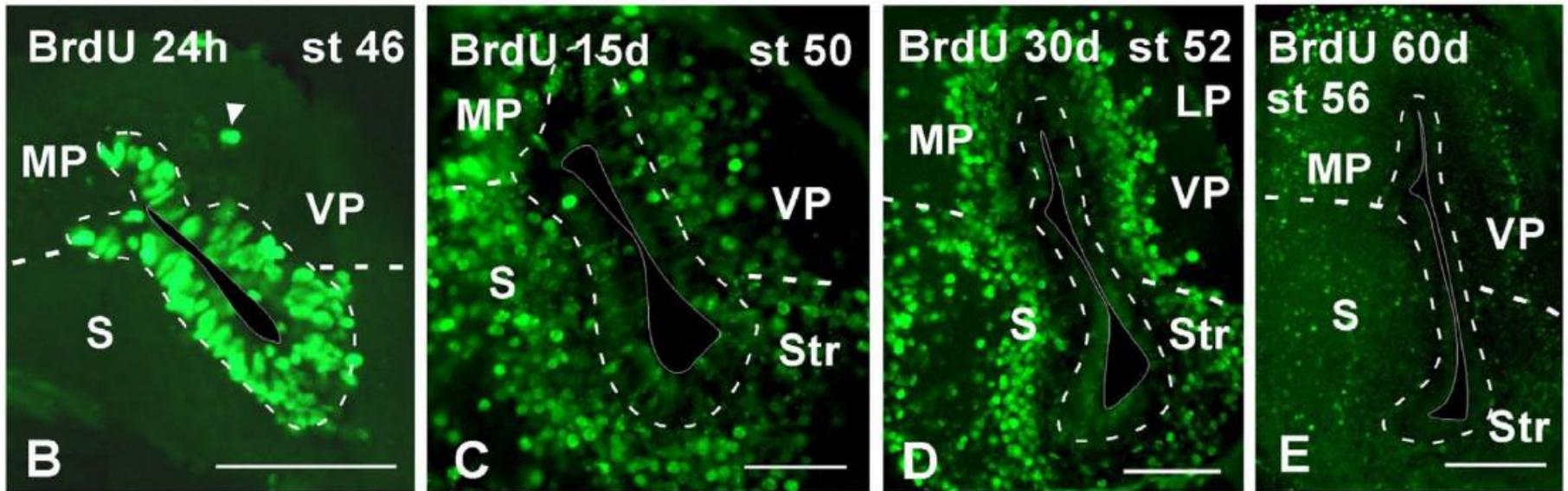
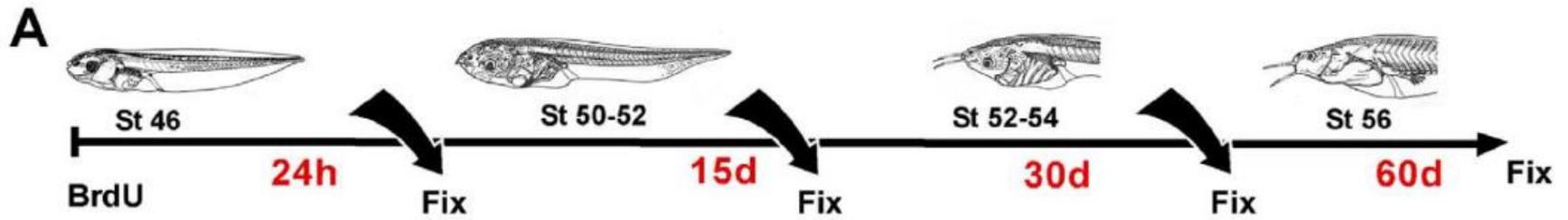
Proliferation analysis with BrdU



st 40 BrdU/PH3/DAPI **st 46 BrdU/PH3/DAPI**



Proliferation analysis with BrdU and neuronal birth



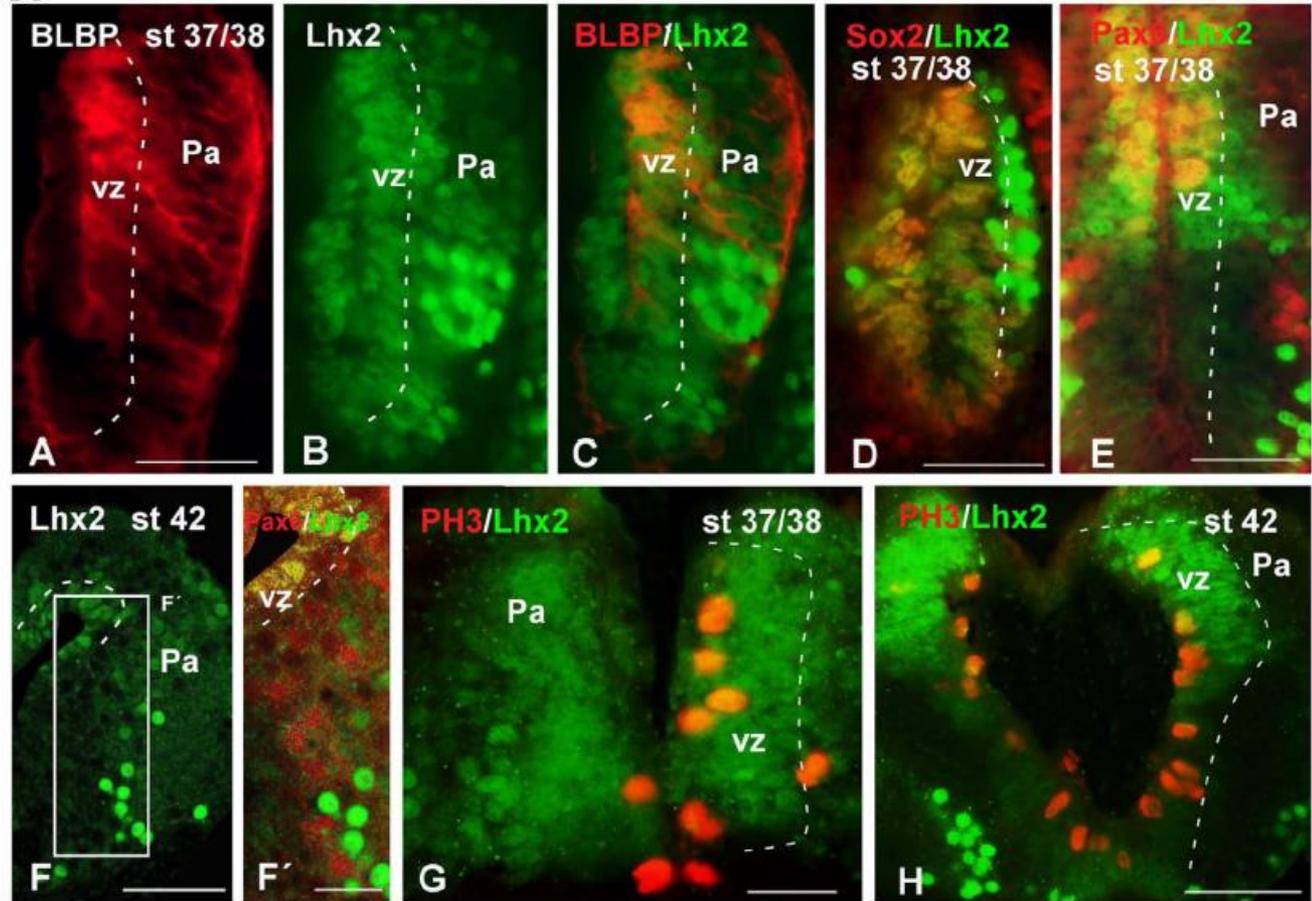
Identification of progenitor cells at embryonic stage: BLBP, Lhx2, Sox2, Pax6

BLBP
Sox2
Pax6

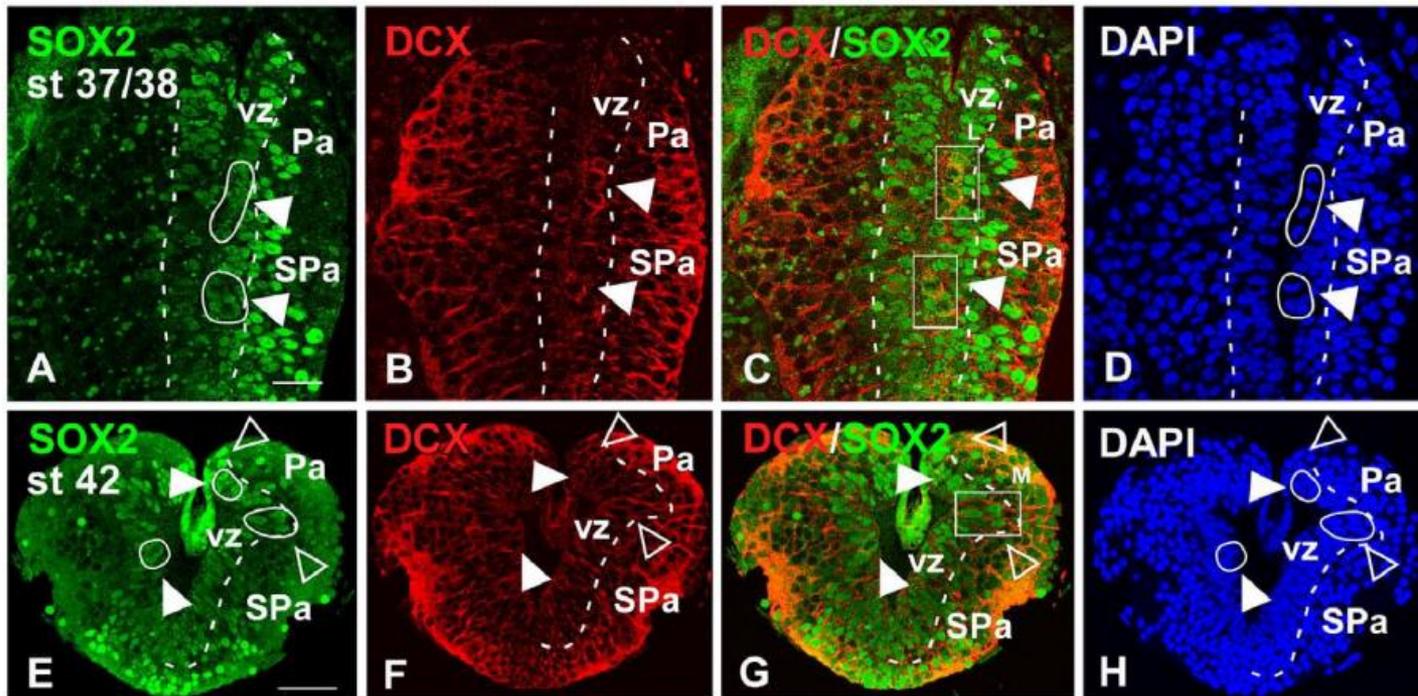
RG cells

Lhx2

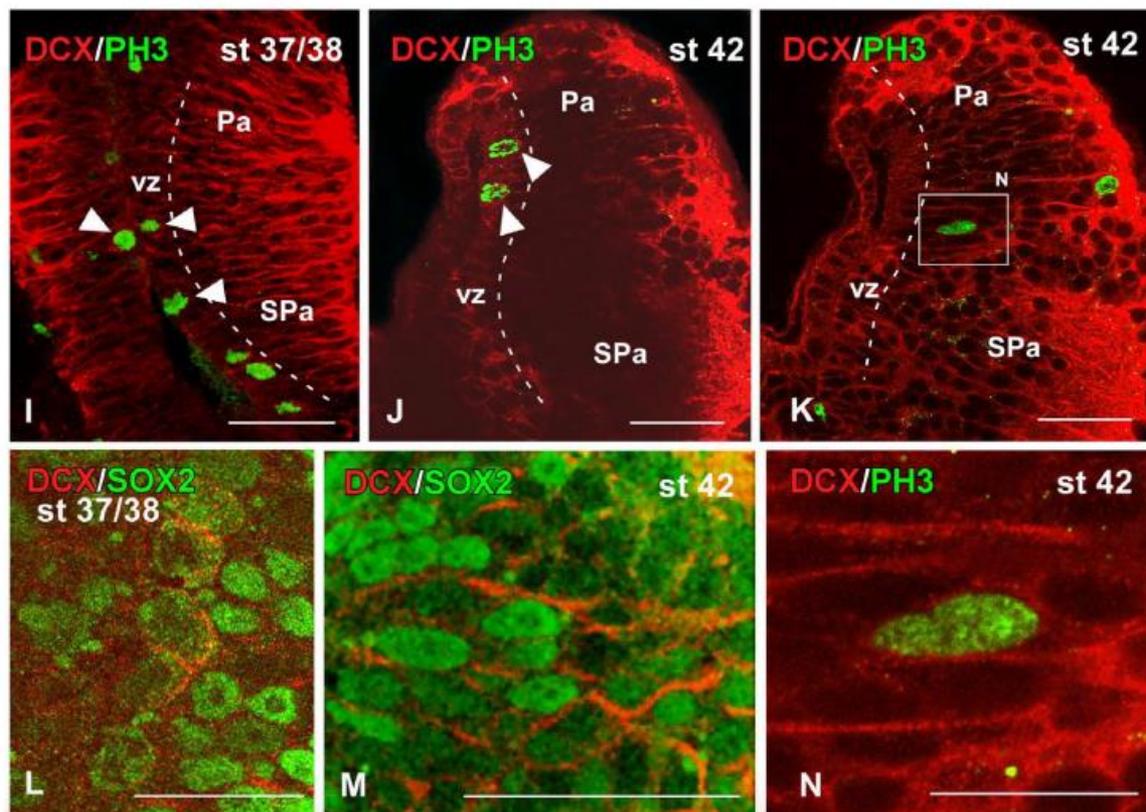
Tbr2
sparse
distribution



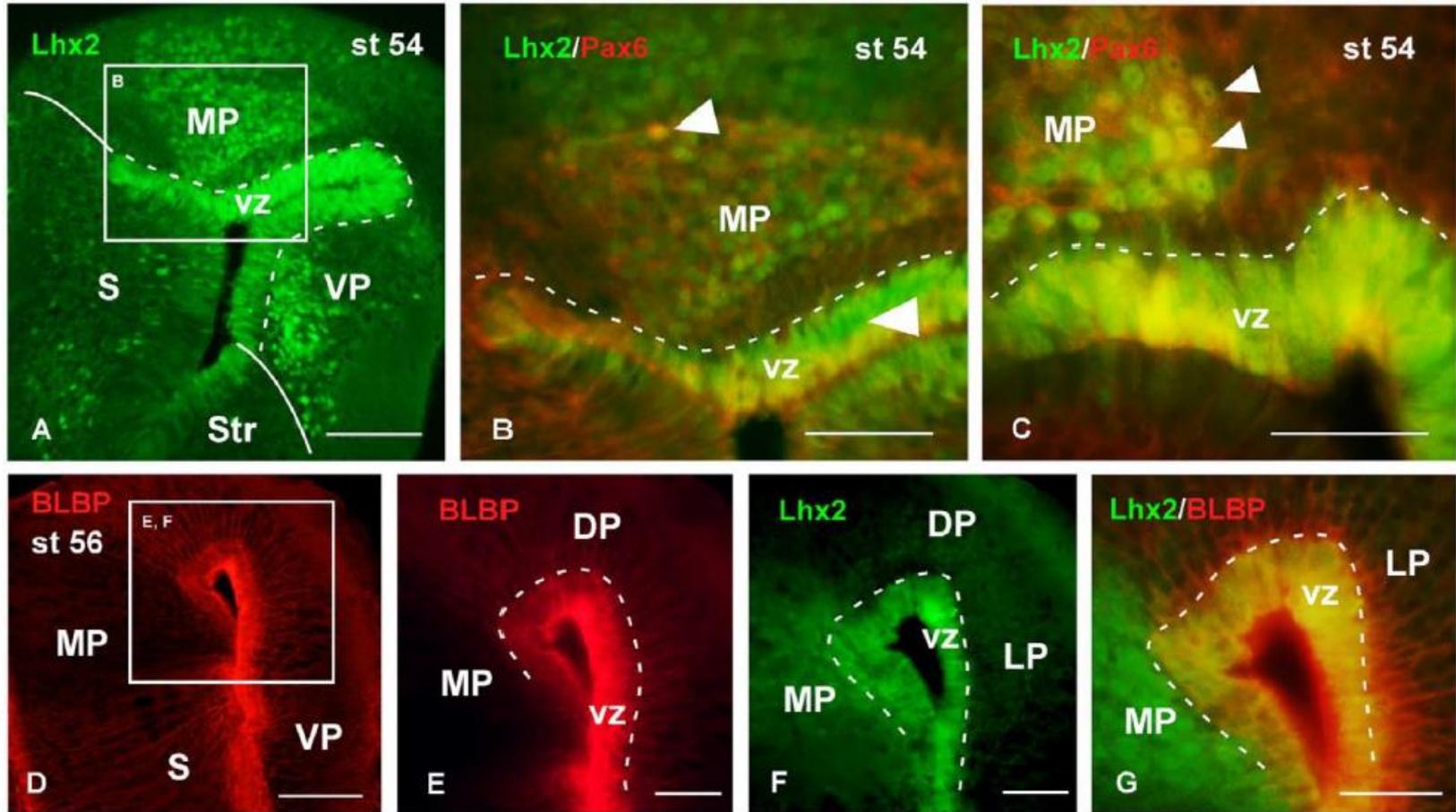
Identification of progenitor cells at embryonic stage: Sox2, Dcx



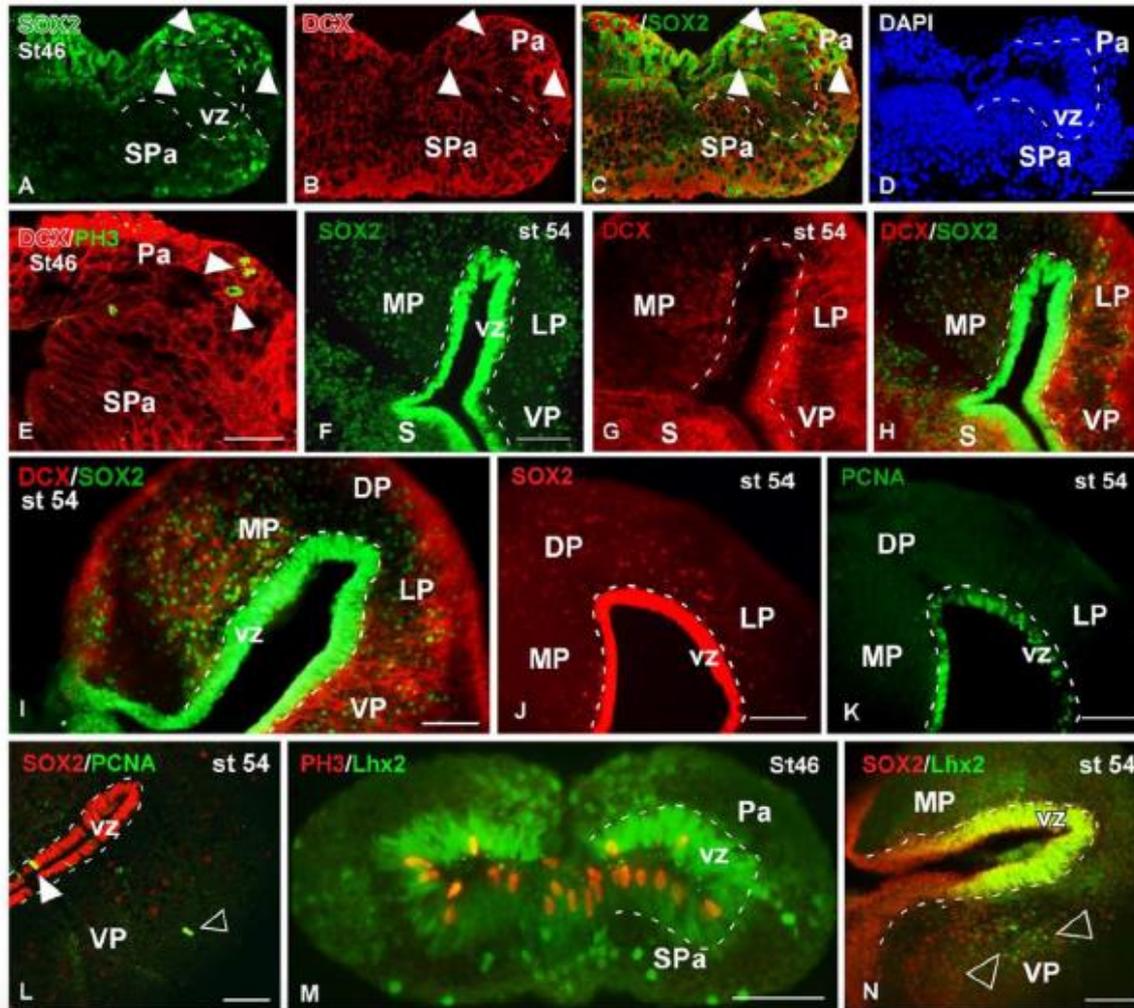
Identification of progenitor cells at embryonic stage: Sox2, Dcx, PH3



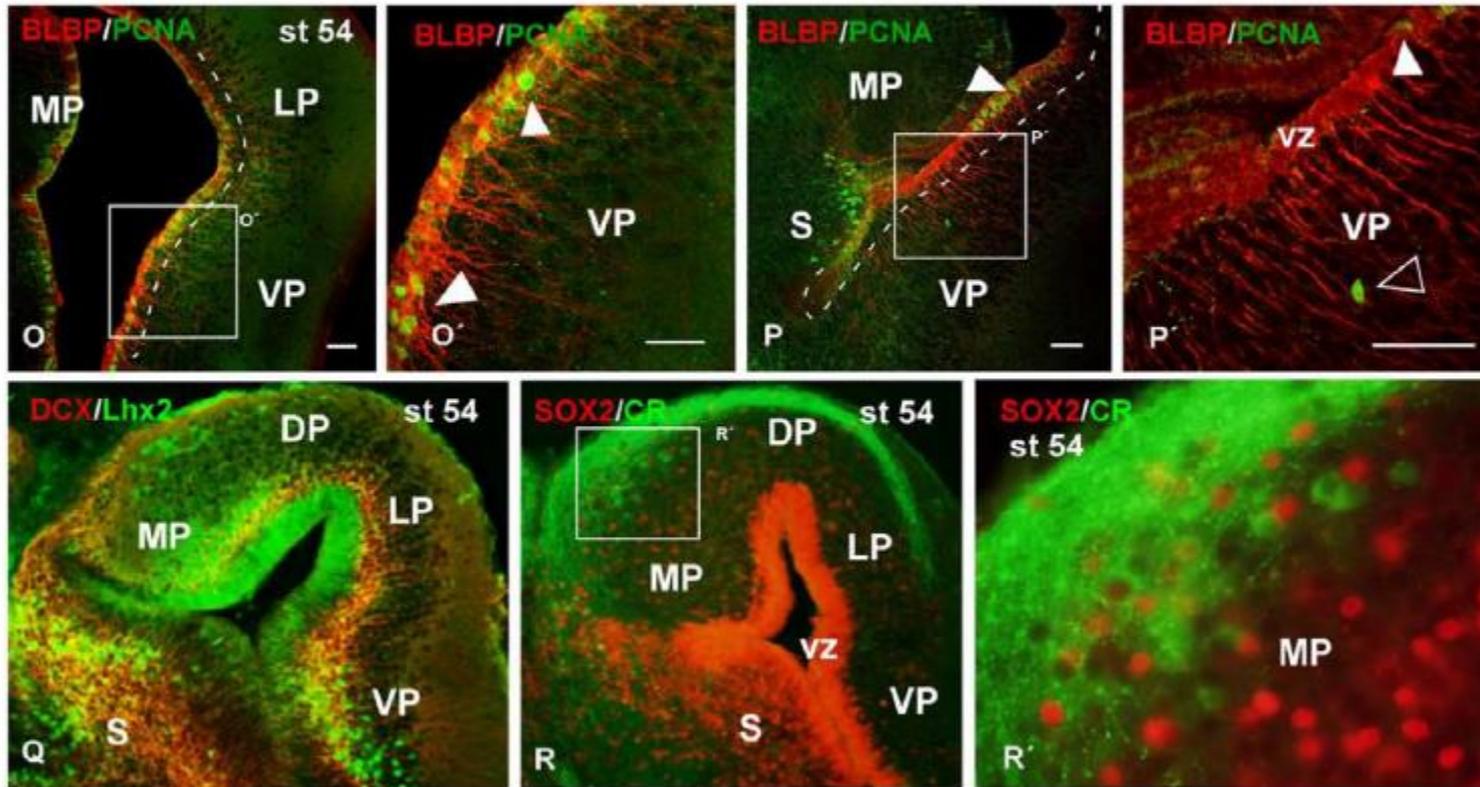
Identification of progenitor cells at larval stage: Lhx2, Pax6, BLBP



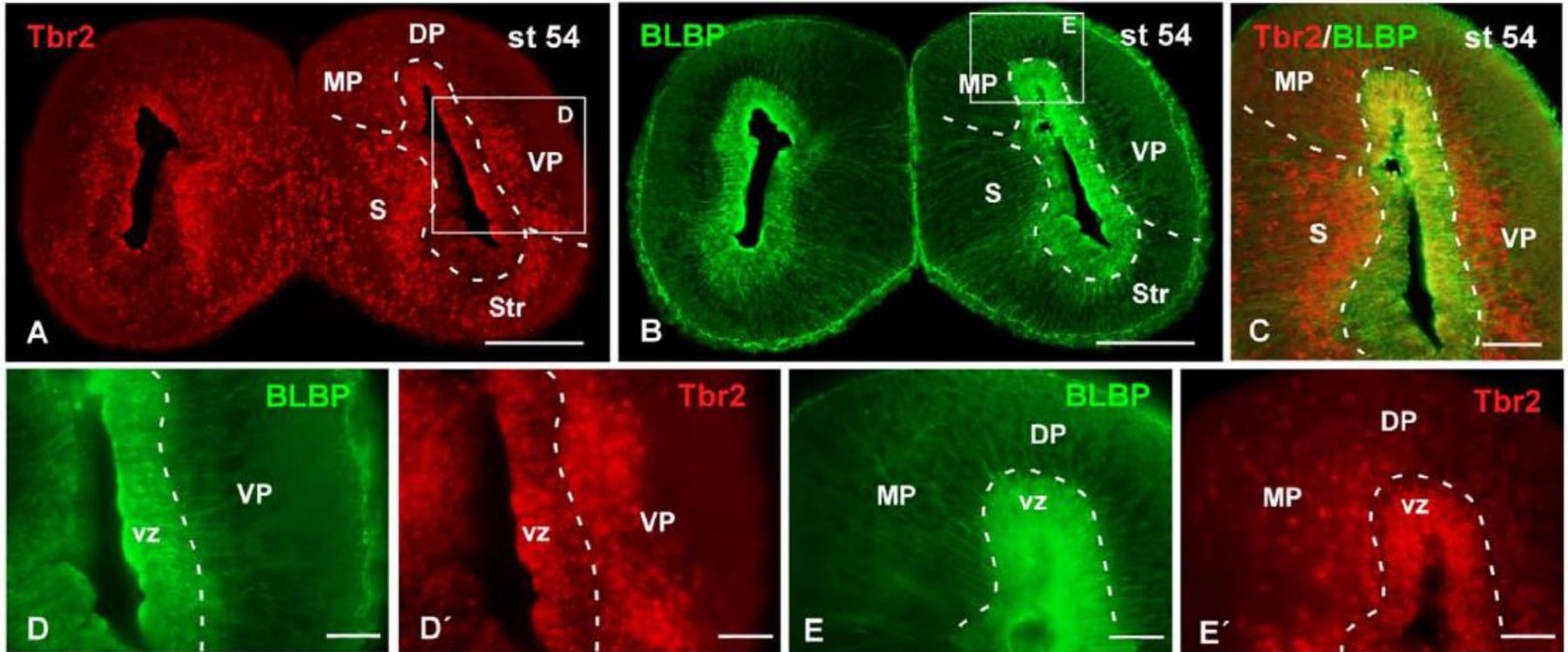
Identification of progenitor cells at larval stage: Sox2, Dcx, BLBP, PCNA, PH3

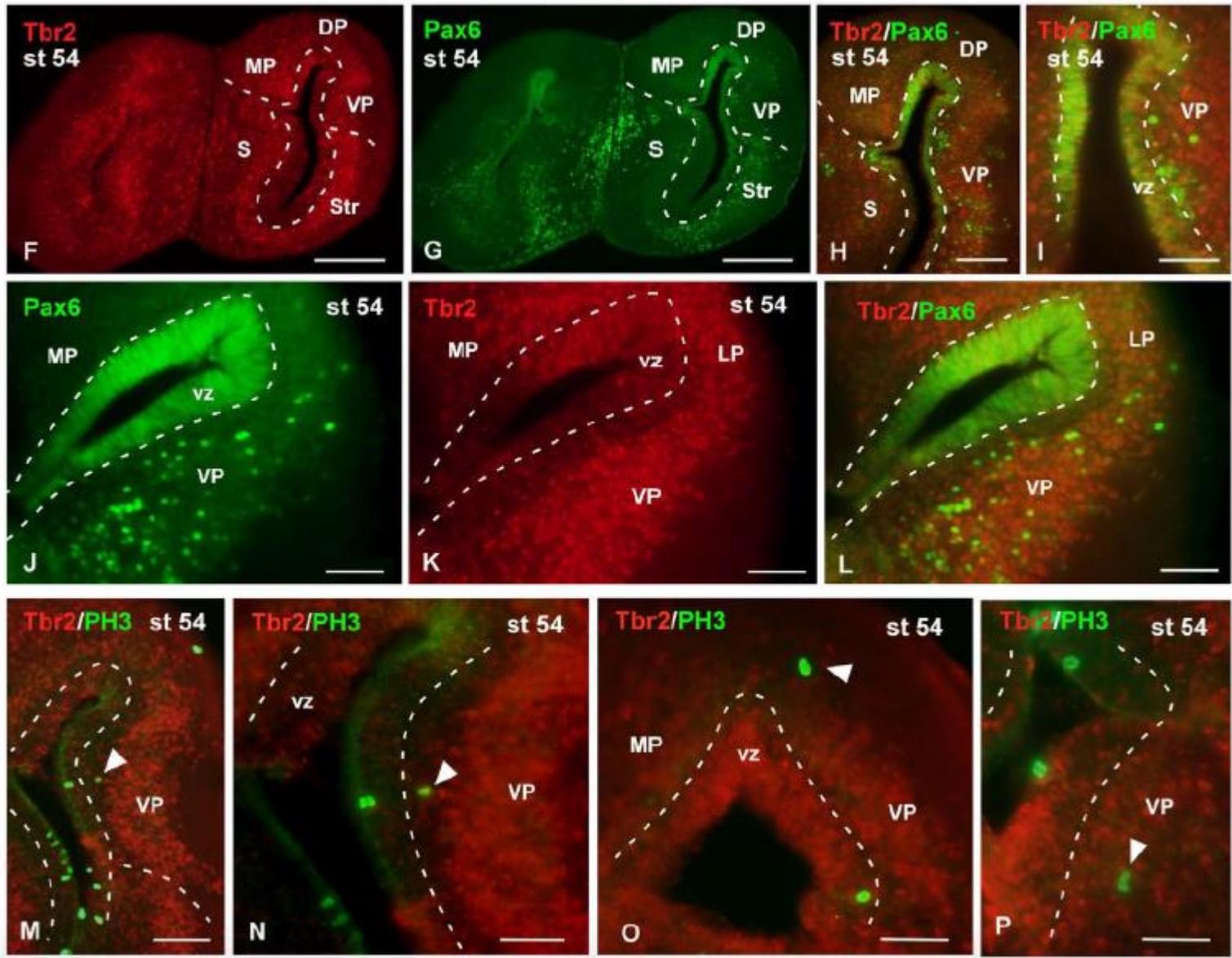


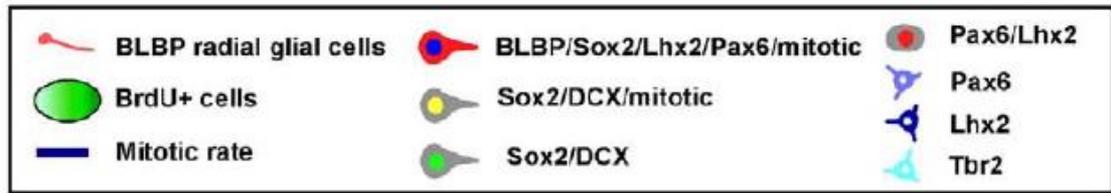
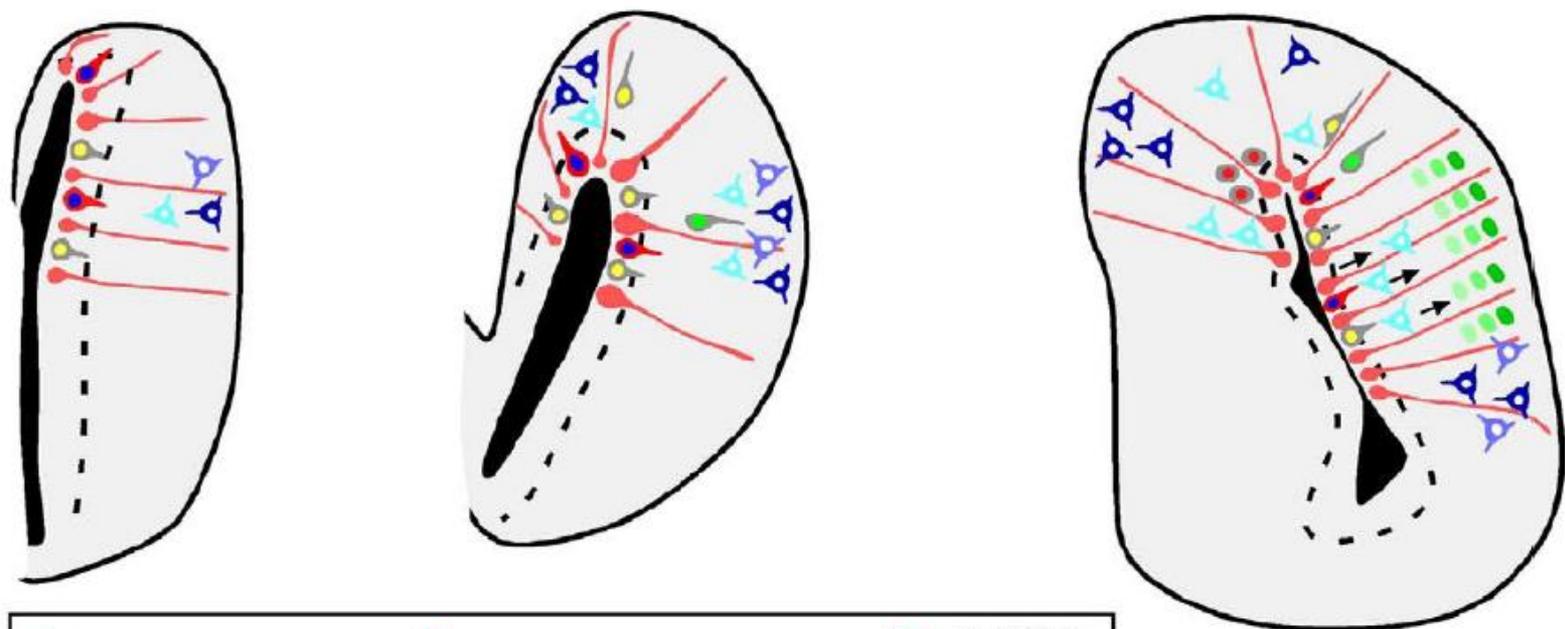
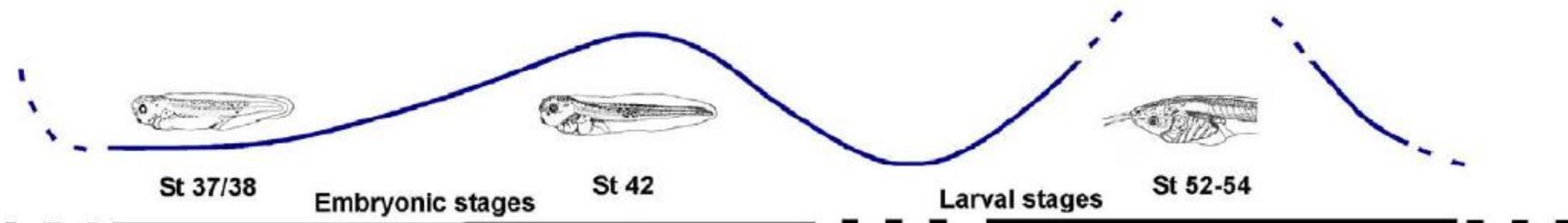
Identification of progenitor cells at larval stage: Sox2, Dcx, BLBP, PCNA, PH3

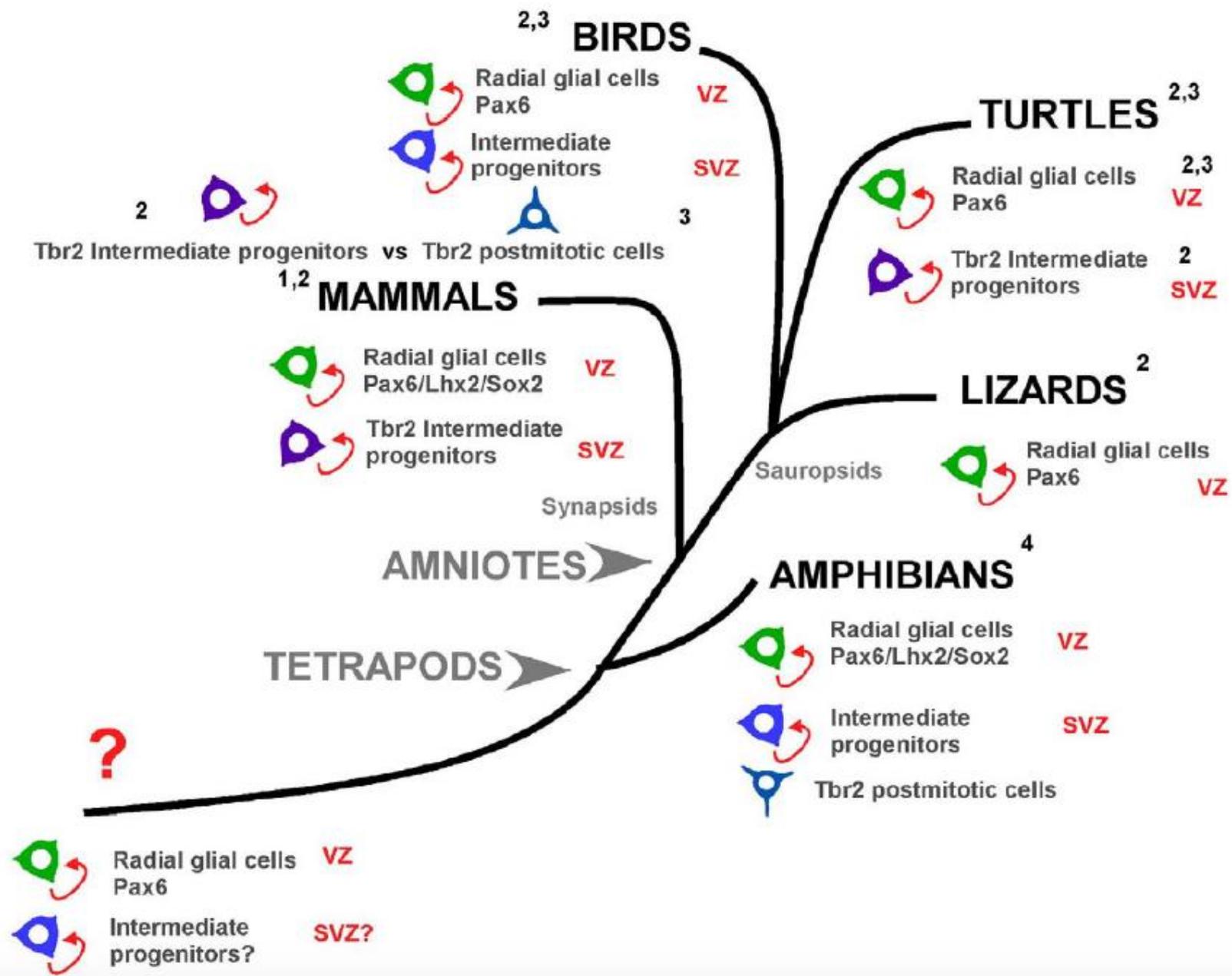


Identification of progenitor cells at larval stage: Tbr2 (SVZ?)















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