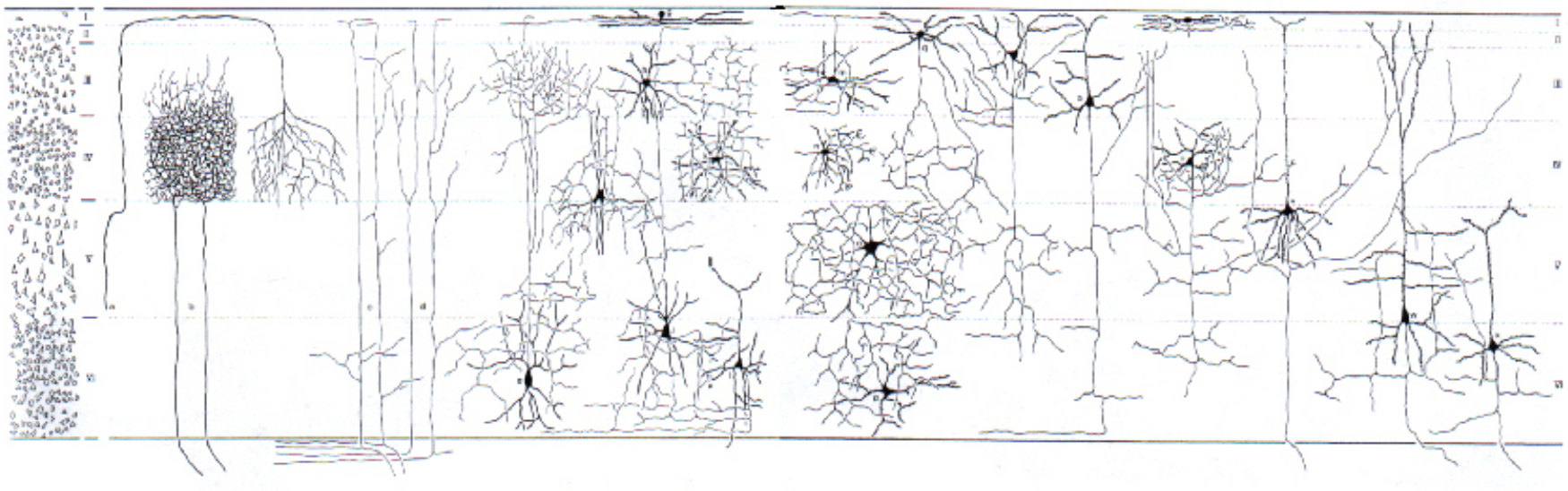
The cerebral cortex: "a master of cellular complexity"

a high degree of neuronal diversity, radially organized into **six layers** and tangentially subdivided into multiple **functional areas**



Encodes and elicits complex cognitive and motor functions

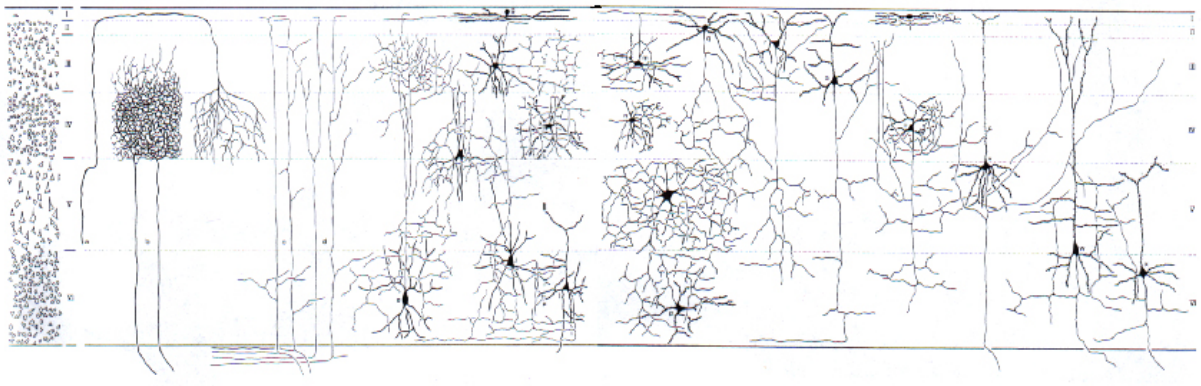
↓

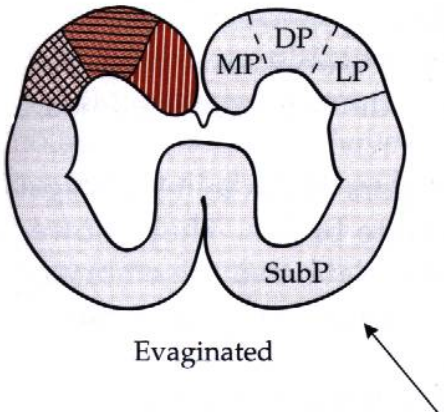
Molecular processes
Neuronal Identity
Connectivity

Established during development

The **neocortex = 6 layers**

I	Molecular layer
II	Outer granular layer
III	Outer pyramidal layer
IV	Inner granular layer
V	Inner pyramidal layer
VI	Multiform layer





" … the original, **primitive pattern** of cortical layering in the whole mammalian class is **six-layered** , and this six-layered pattern is visible in all orders, either **permanently** or at least as a **temporary ontogenic stage** in the embryo"

Brodmann 1909

2 main classes of neurons

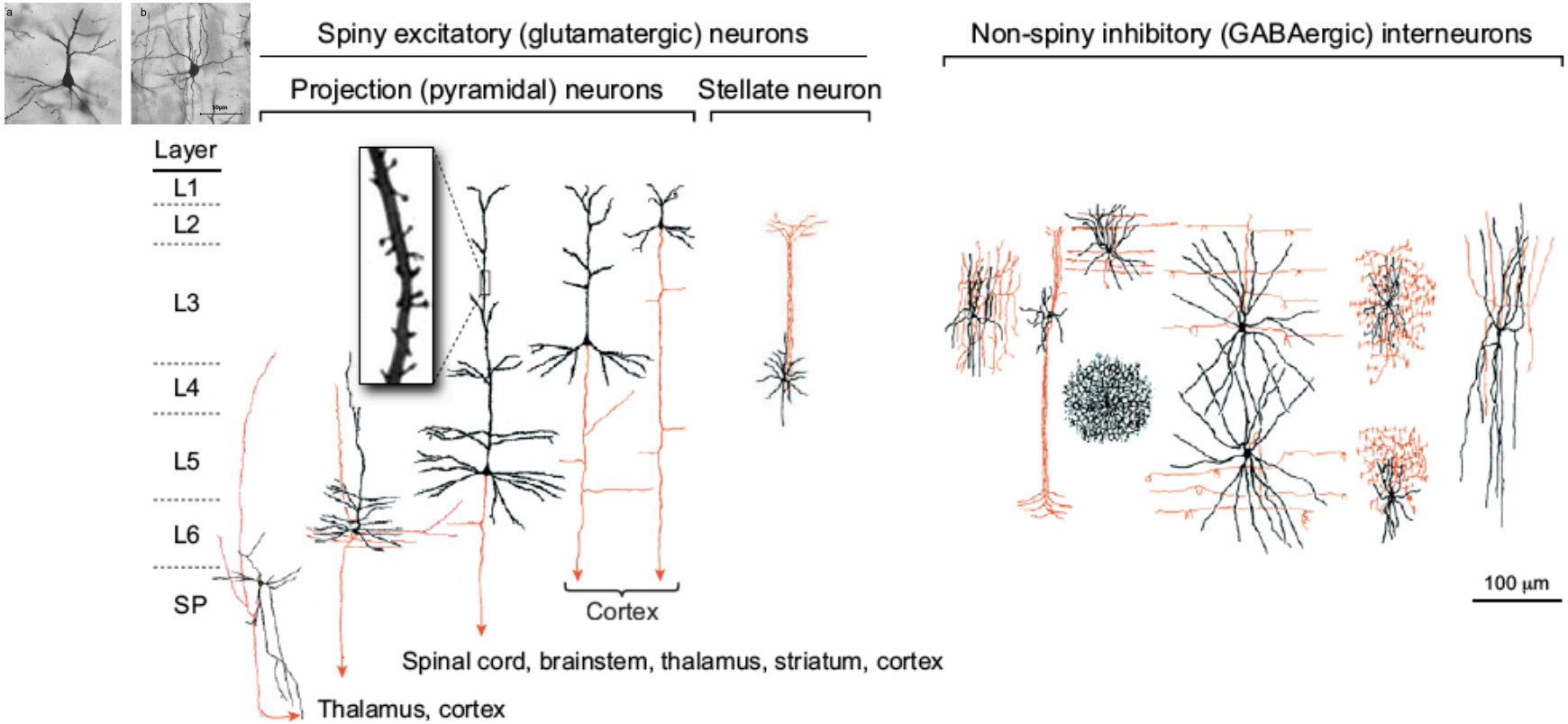
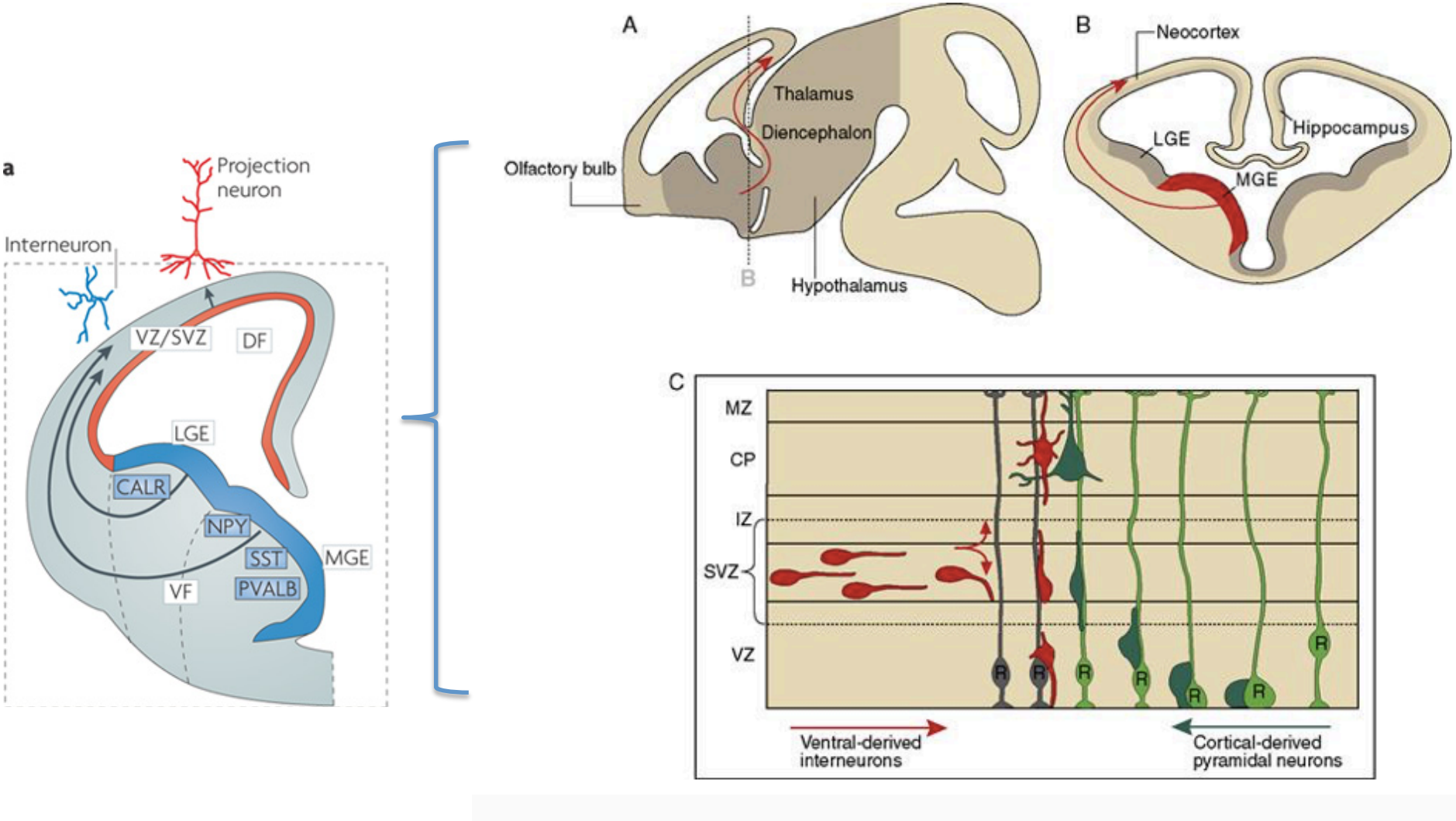


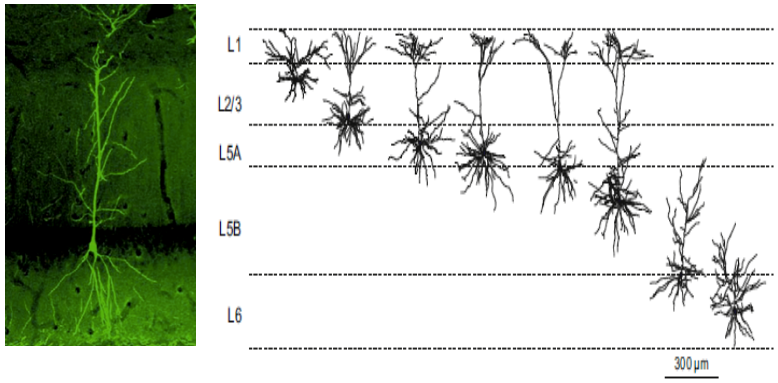
Fig. 1. Major neuronal cell types of the adult cerebral cortex. Cortical neurons (shown here for primates) are categorized into two major classes: spiny excitatory (glutamatergic) neurons (left panel) and non-spiny inhibitory (GABAergic) interneurons (right panel). The former, the dendrites of which are decorated by numerous post-synaptic membranous protrusions termed spines (see inset), include the projection (pyramidal) neurons, the principal cells of the neocortex, and stellate neurons (see Glossary, Box 1), which are mostly found in L4 of primary sensory areas. Projection neurons display marked layer- and subtype-specific differences in the morphology of their dendrites (black) and in the targets of their axonal projections (red); neurons within the deep layers (L5 and L6) and the subplate (SP) project axons that target cortex and subcortical structures, including striatum, thalamus, brainstem and spinal cord, whereas upper-layer (L2-L4) neurons project axons within the cortex. The non-spiny interneurons, which are highly diverse in morphology, neurochemistry and electrophysiology, project axons within a local circuit. Subtypes of interneurons also display laminar preferences, thereby contributing to layer differences in cortical circuitry. Adapted from Jones (Jones, 1986).

2 main classes of neurons: DIFFERENT ORIGIN

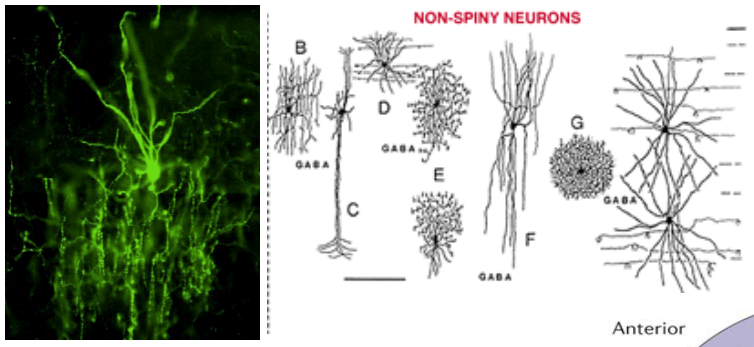


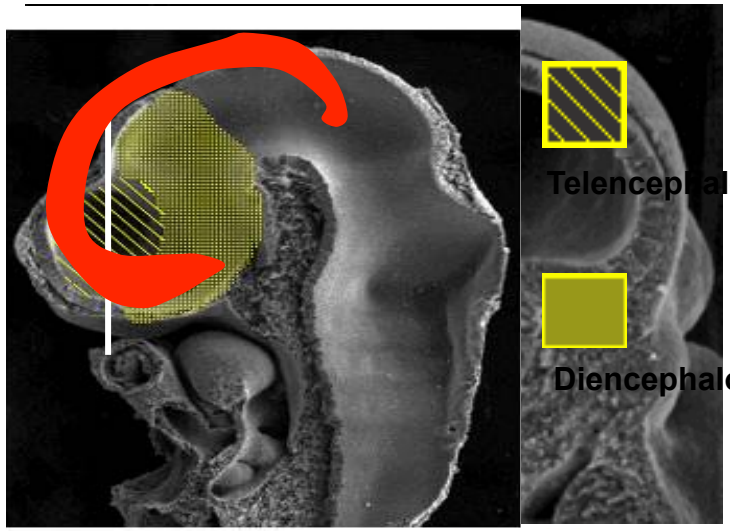
Cortical Projection neurons and Interneurons are born from different D/V regions of the telencephalon

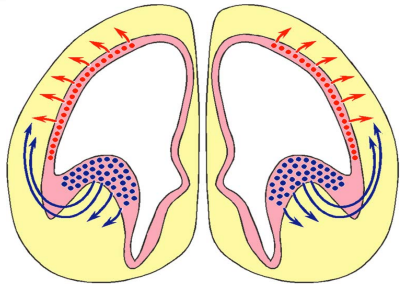
Glutamatergic projection neurons

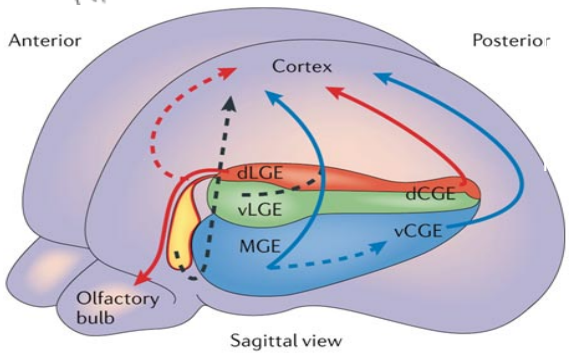


GABAergic cortical interneurons









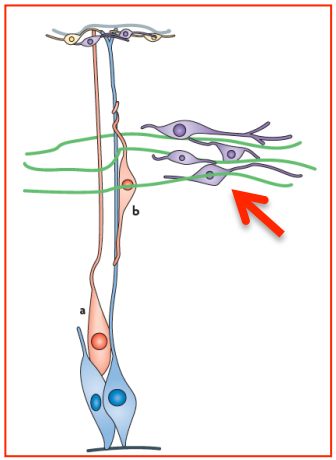
Dorsal (pallium)

Ventral (subpallium)

Interneurons

Origin: subpallium

Tangential migration



Ghashghaei and Anton, 2007

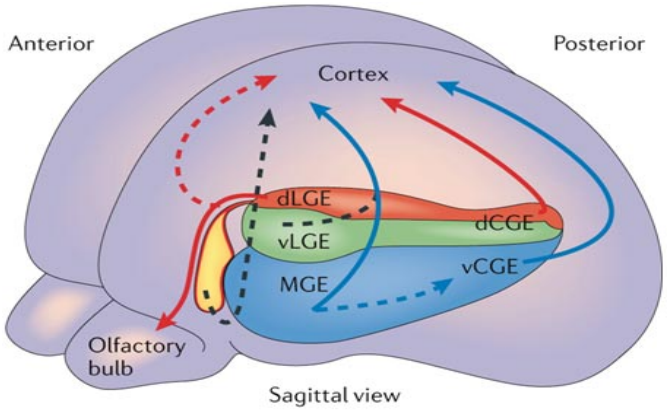
NUMBER: 20-30% of the cortical neuronal population

GABA-ergic

FUNCTION: control and synchronize the output of pyramidal neurons

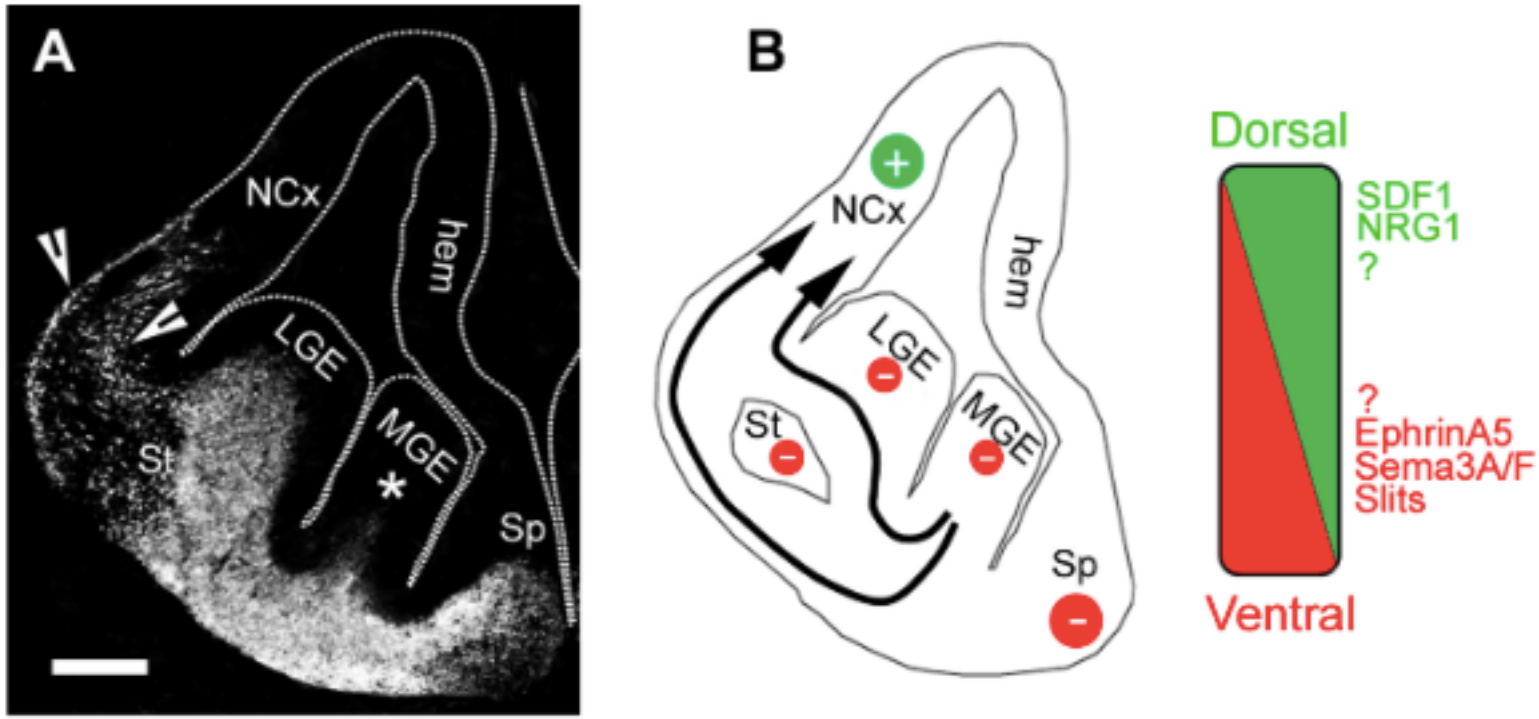
SUBTYPES: several subtypes (morphology, molecular, targeting, synaptic trasmission, physiological character)

DYSFUNCTION: disruption of GABAergic inputs to pyramidal cells



Generation of the different subpopulations of cortical interneurons is linked to regional differences in the specification of progenitor cells in the subpallium

expression of particular combinations of transcription factors

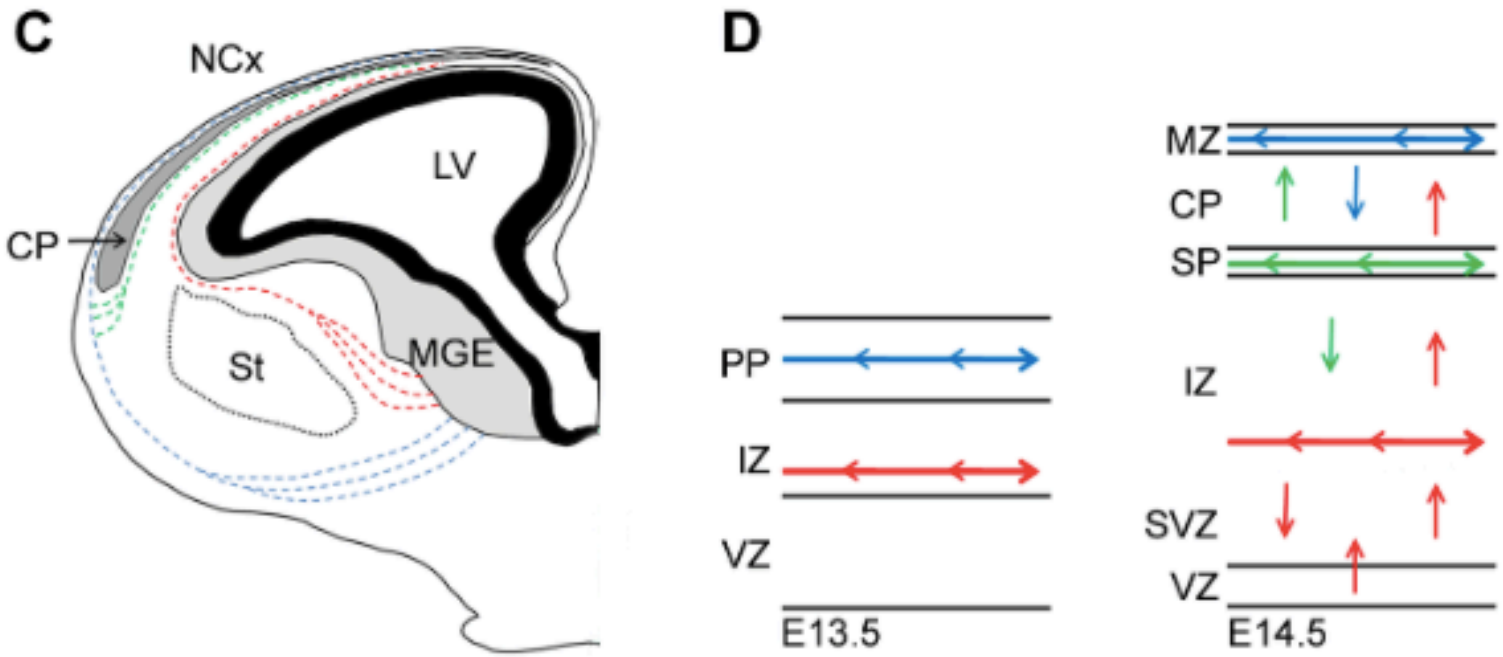


Tangential migration of cortical interneuron

(A) Coronal section taken from the brain of an E13.5 GAD67–GFP (green fluorescent protein) transgenic mouse.

(B) Schematic drawing of (A) illustrating chemorepulsive (red) and chemoattractive (green) gradients established from ventro-dorsal and dorso-ventral parts of the forebrain respectively.

Hernandez-Miranda et al., 2010



Coronal section of E15 mouse brain showing the tangential paths of **early- (blue** broken lines), and **late (red** broken lines)-born interneurons. Upon the emergence of the CP, an additional migratory path is formed within the subplate (green broken lines).

(D) tangential and radial movements of interneurons within the cortical wall at E13.5 and E14.5.

LV, lateral ventricle; PP, preplate; NCx, neocortex; SP, subplate; St, striatum

Hernandez-Miranda et al., 2010

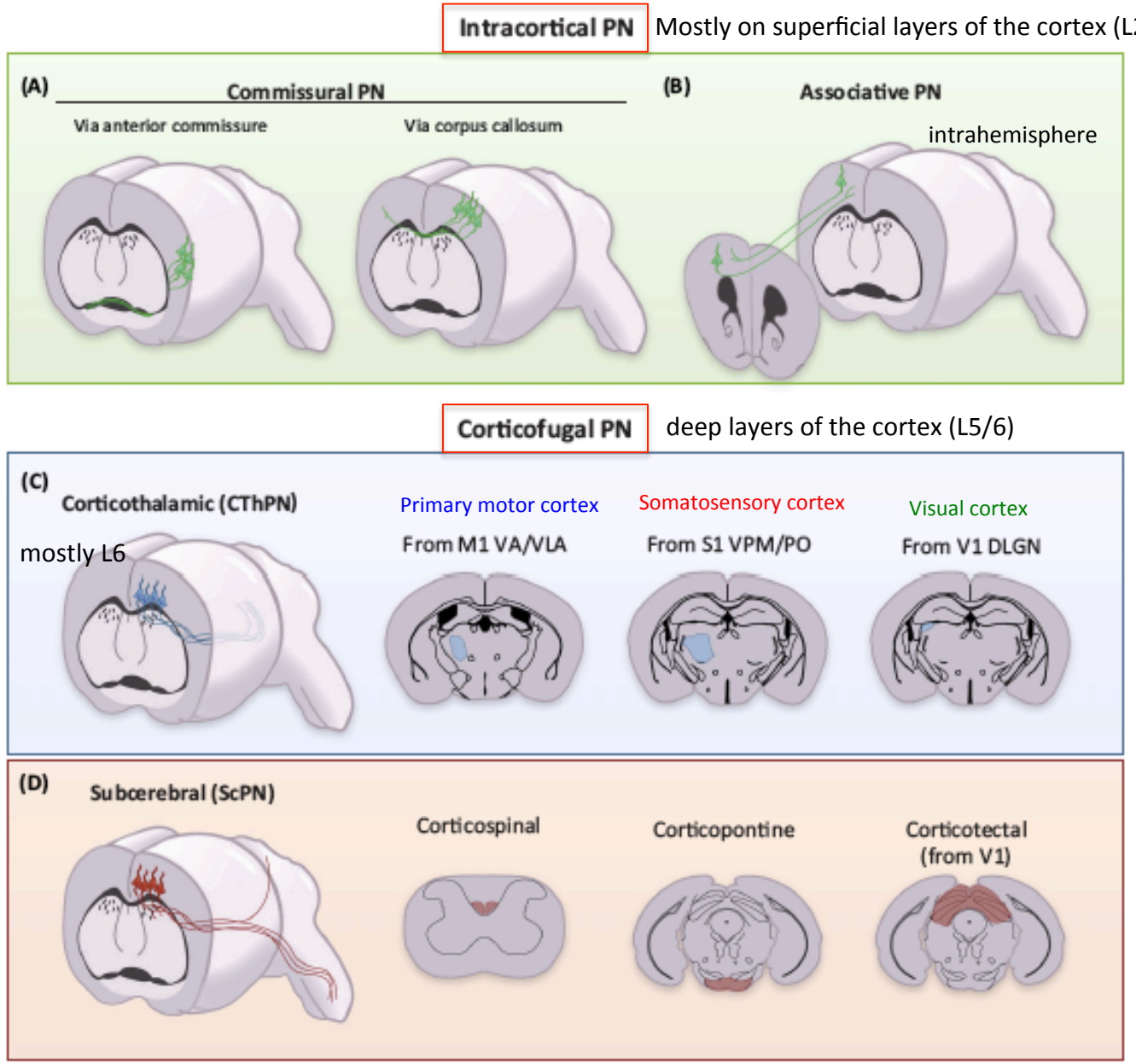
Pyramidal neurons = 70-80% cortical neuronal population

Classification:

- ✓ Laminar position
- ✓ Morphology
- ✓ Molecular Identity (combinatorial code)
- ✓ Electrophysiological profile
- ✓ **Connectivity**

PN with collaterals → Sending axons to multiple targets

VA=ventral anterior
VLA=anterior ventral lateral
VPM=ventral posterior medial nucleus
PO=posterior nucleus
dLGN=dorsal lateral geniculate nucleus



Lodato et al., 2014

TRENDS in Neurosciences

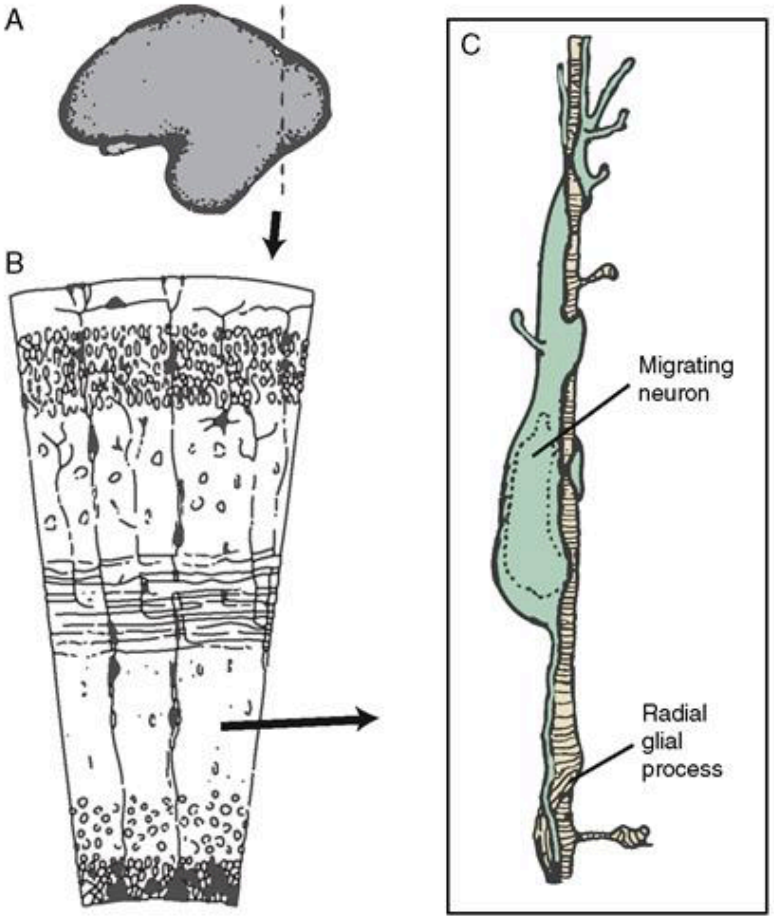
The emergence of laminar-dependent patterns of functional neocortical connectivity depends on **two crucial** early **developmental processes**:

1) the **generation** and **migration** of neurons to their final position within the nascent neocortex;

2) the acquisition of **distinct neuronal identities** and connections

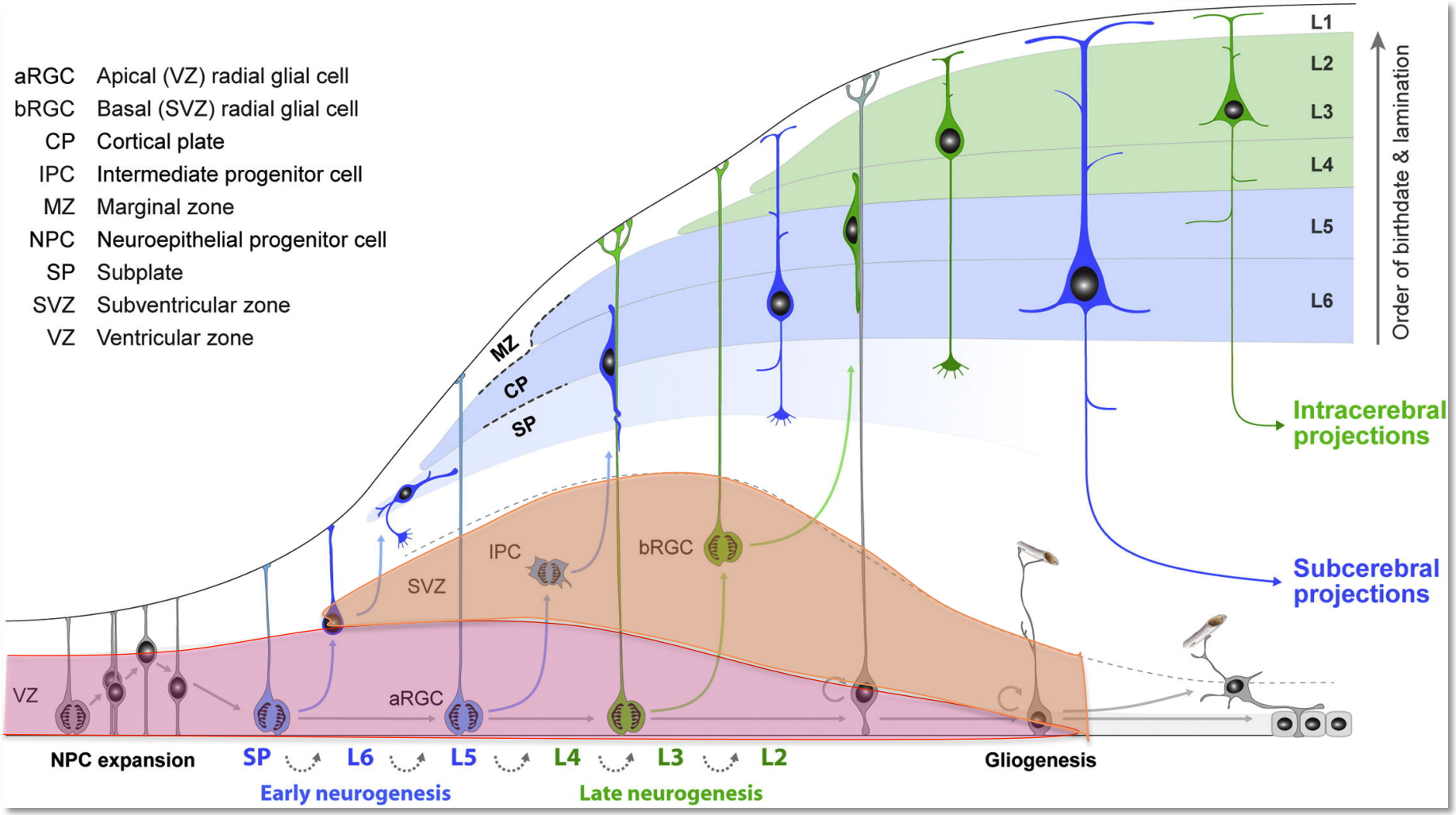
→ control of neuronal positioning and acquisition of laminar and projectional identity by **cell type- and layer-specific transcriptional programs**

Cerebral cortex histogenesis : glial-guided neuronal motility



- cell-cell interactions,
- cytoskeletal dynamics,
- leading process extension,
- somal translocation
- migration termination

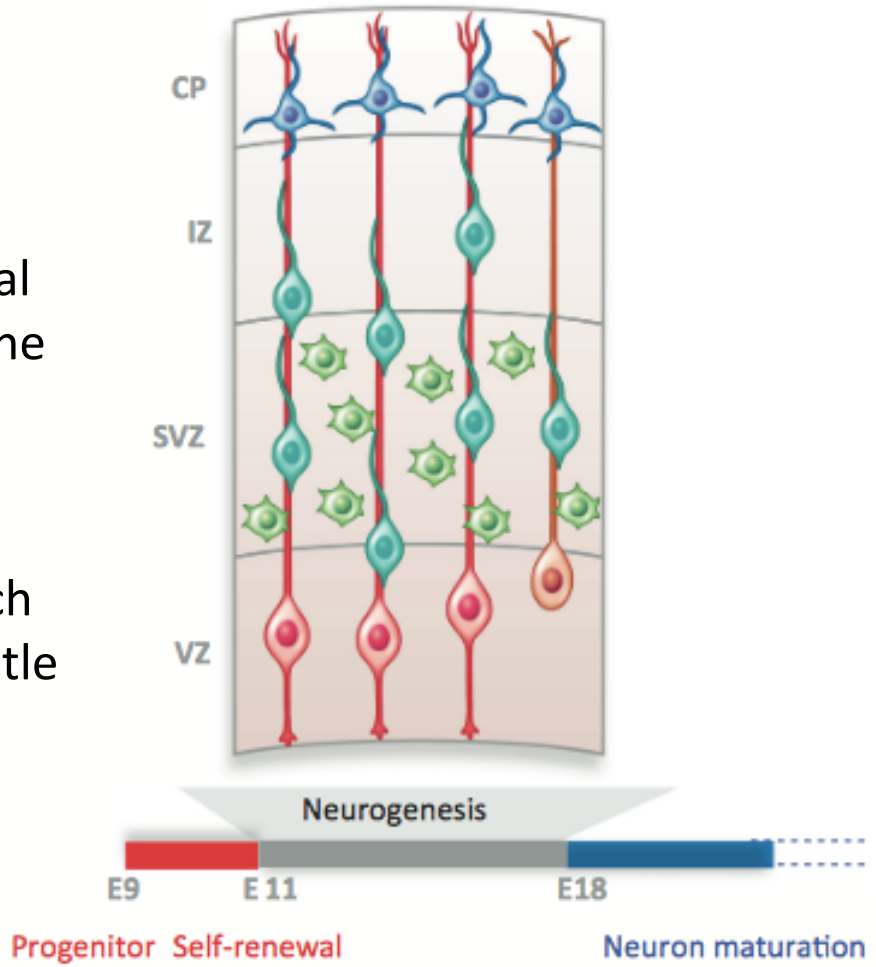
Mammalian corticogenesis



modified from Kwan et al., 2012

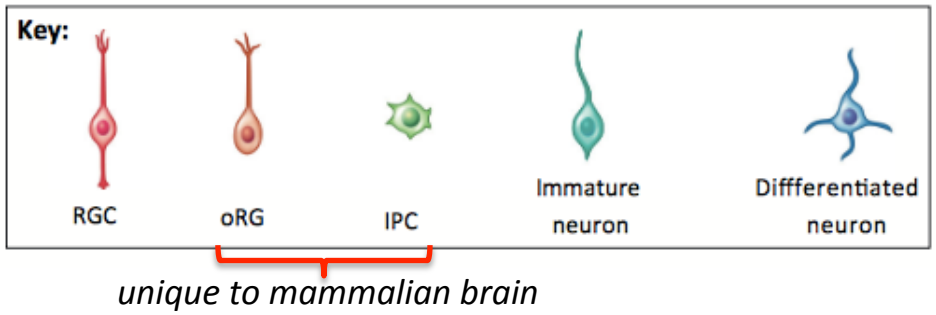
Cortical projection neurons:

1) are born in the dorsal germinal zones of the telencephalon, the ventricular (VZ) and subventricular (SVZ) zones
2) migrate through the intermediate zone (IZ) to reach their final position in the mantle layers

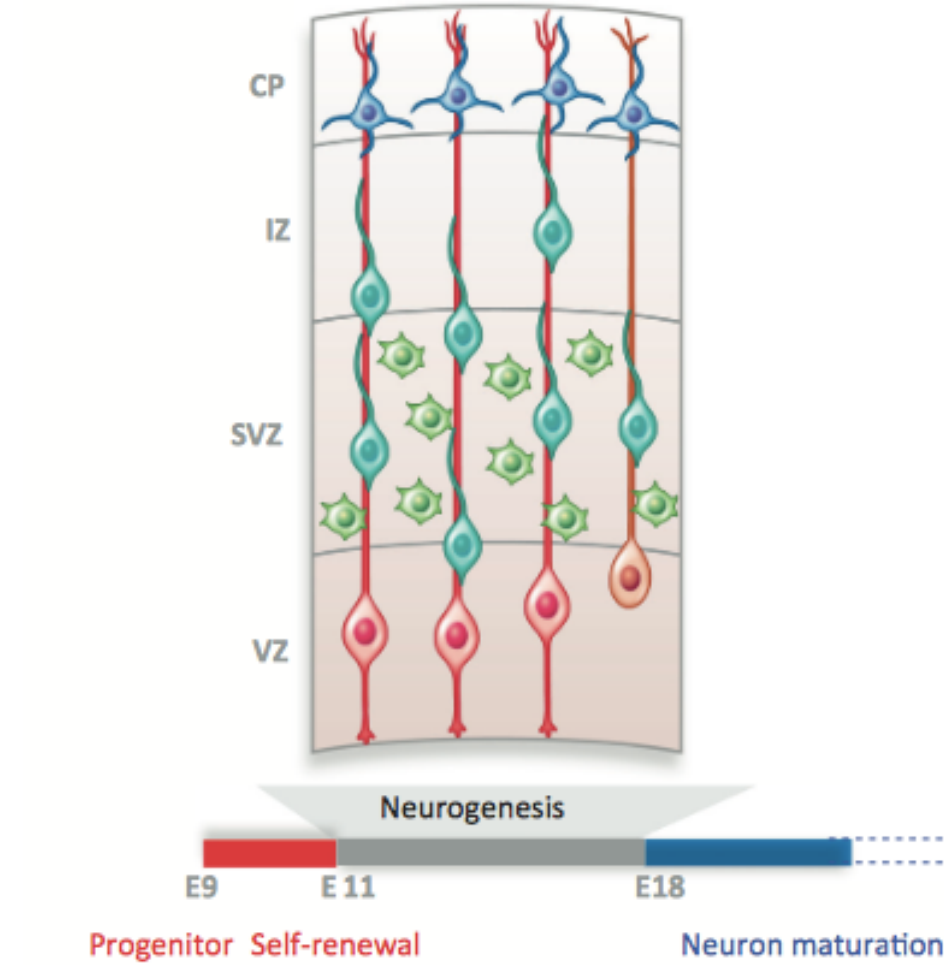


Mouse

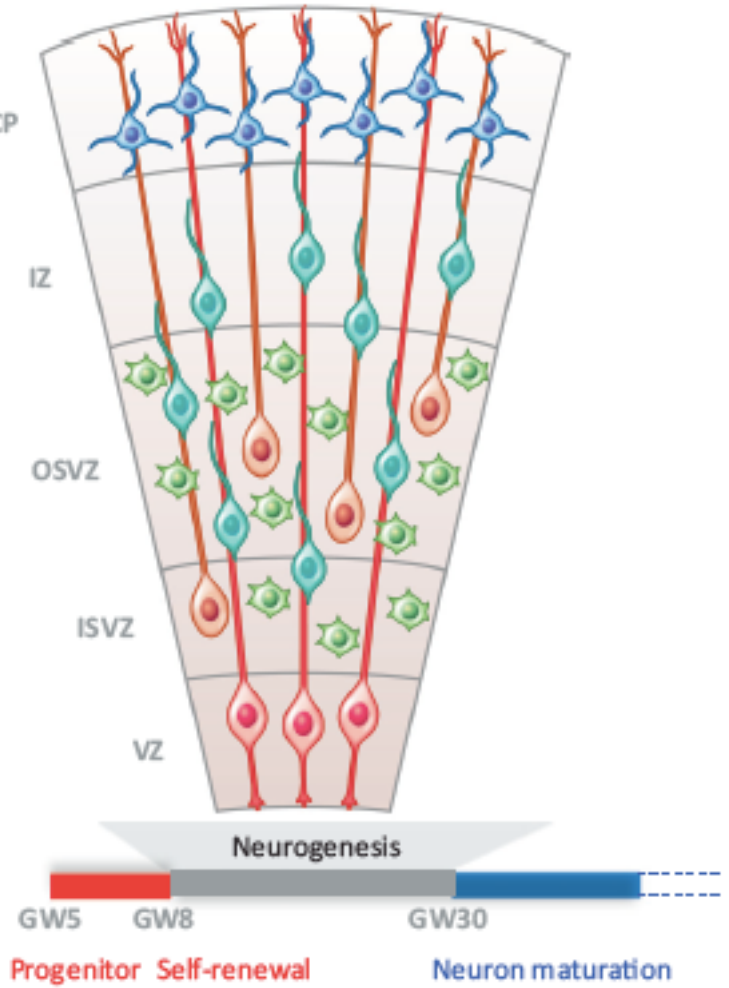
RGC=radial glial cell
oRG=outer radial glial cell
IPC= intermediate precursor cell



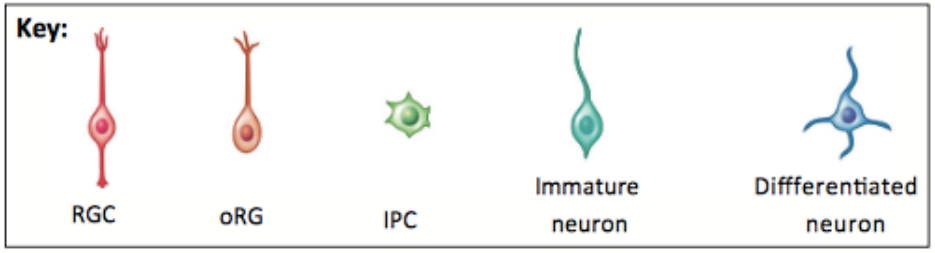
unique to mammalian brain


CP
IZ
SVZ
VZ
Neurogenesis
E9
E 11
E18
Progenitor Self-renewal
Neuron maturation

Mouse


CP
IZ
OSVZ
ISVZ
VZ
Neurogenesis
GW5
GW8
GW30
Progenitor Self-renewal
Neuron maturation

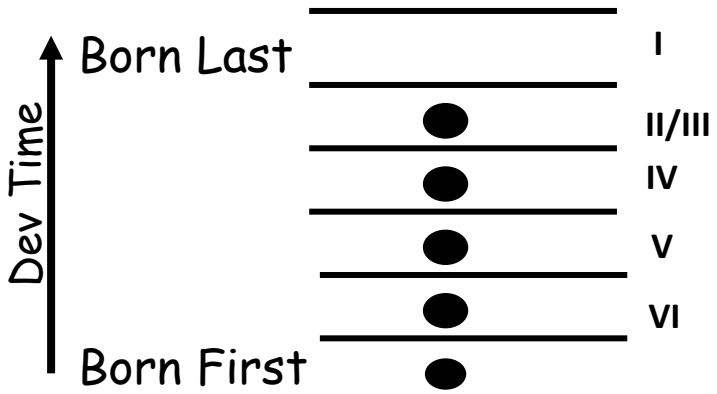
Human

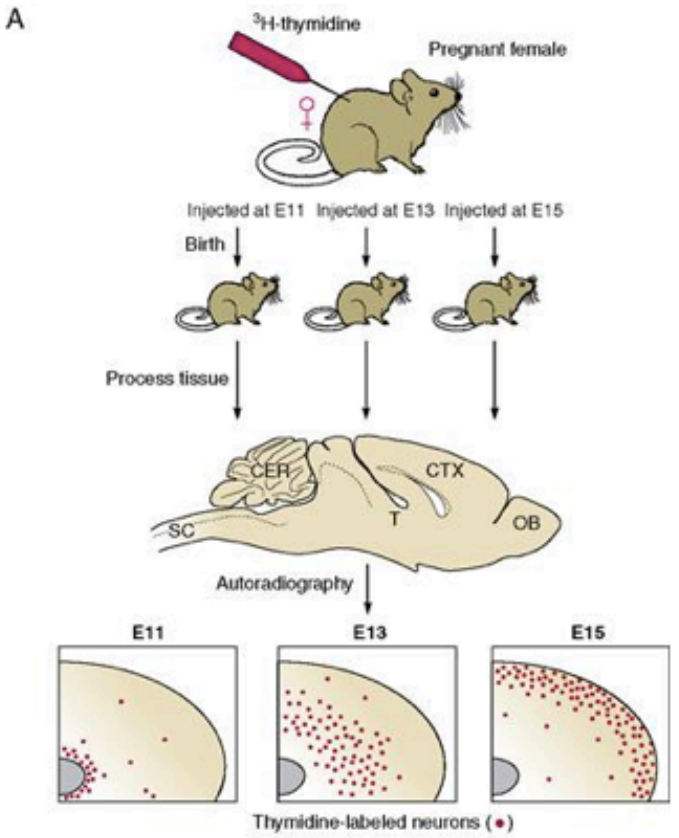

Key:
RGC
oRG
IPC
Immature neuron
Diffferentiated neuron

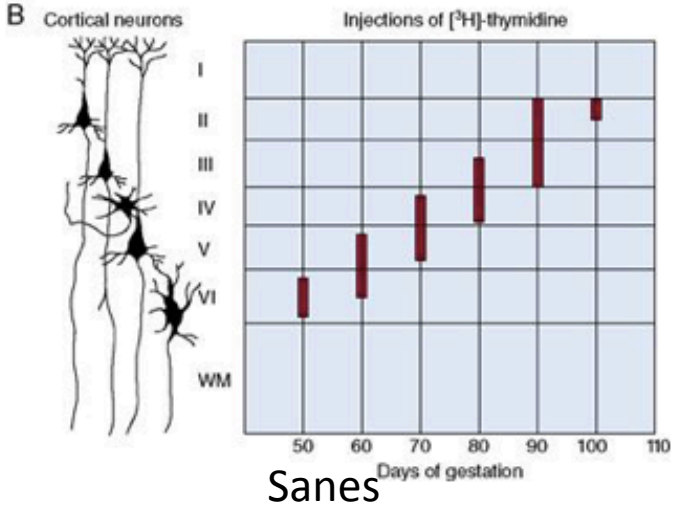
Inside-out pattern

“early studies”

As neurogenesis progresses, diverse subtypes of projection neurons are generated sequentially and their migration into the mantle layers occurs in an inside-first, outside-last manner

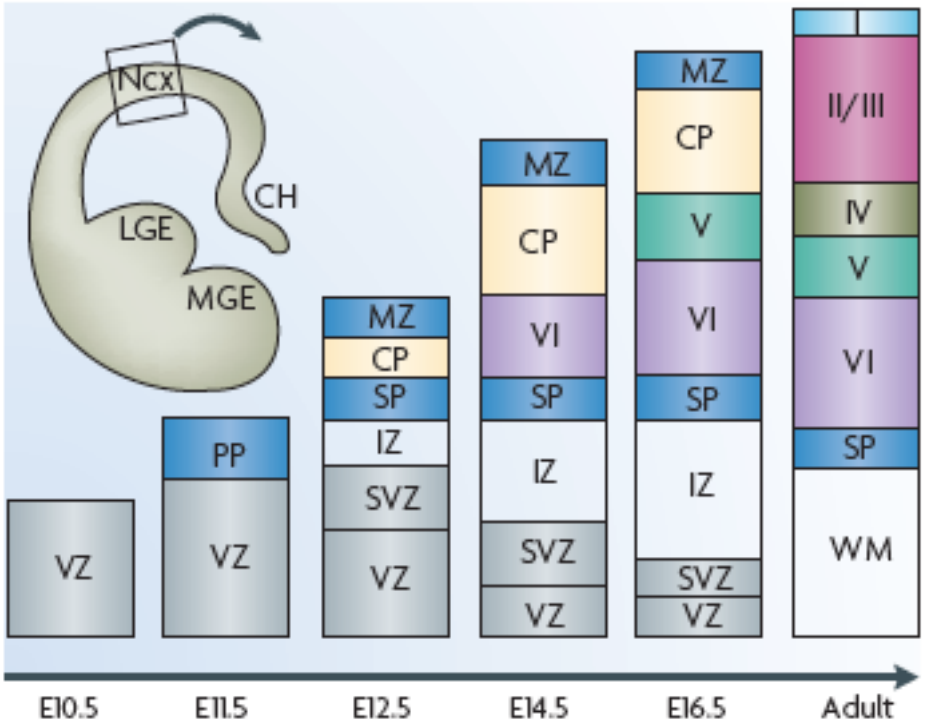






Sanes

Inside-out pattern

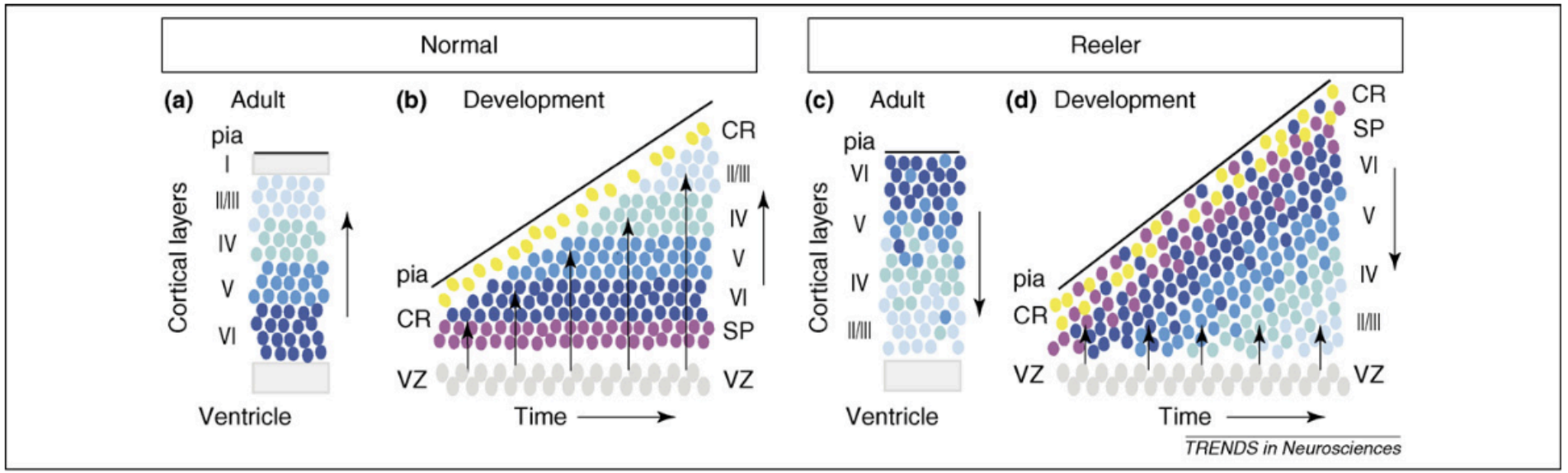


Progenitors residing in the VZ and SVZ in mice produce projection neurons in an 'inside-out' fashion.

The earliest born neurons form the preplate (PP), which is later split into the more superficial marginal zone (MZ) and the deeply located subplate (SP). The cortical plate (CP), which will give rise to the multilayered neocortex, develops in between these two layers.

CH, cortical hem; Ncx, neocortex; IZ, intermediate zone; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; SVZ, subventricular zone; VZ, ventricular zone; WM, white matter.

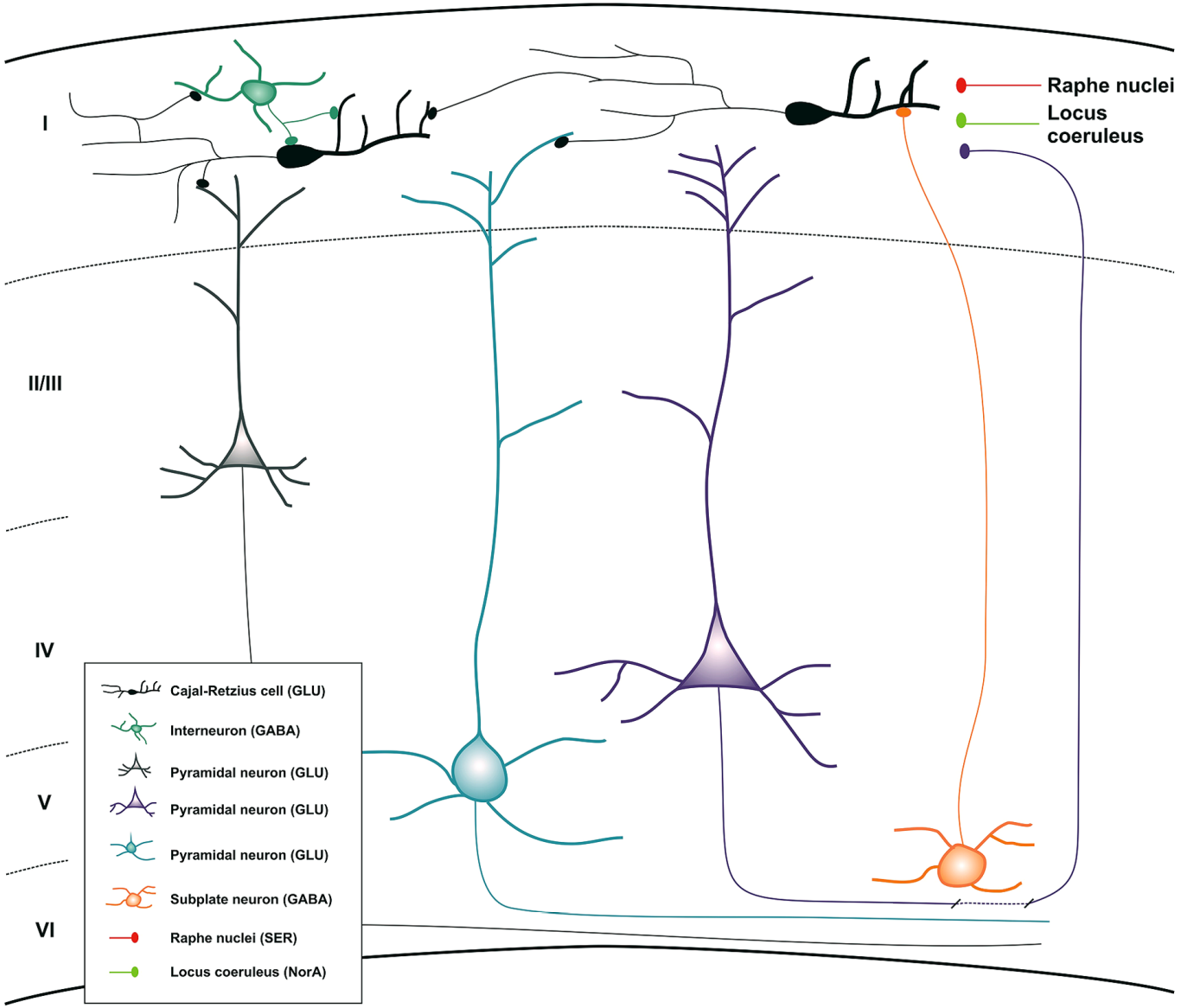
Reeler mutant mice
(spontaneous mutation - autosomal – recessive)



*In the **spontaneous mouse mutant Reeler**, cortical neurons are generated normally but they are misplaced – the cortical layers are approximately inverted with respect to birth order - the layers are disordered, and the orientation of the neurons is less precise than in the wild type*

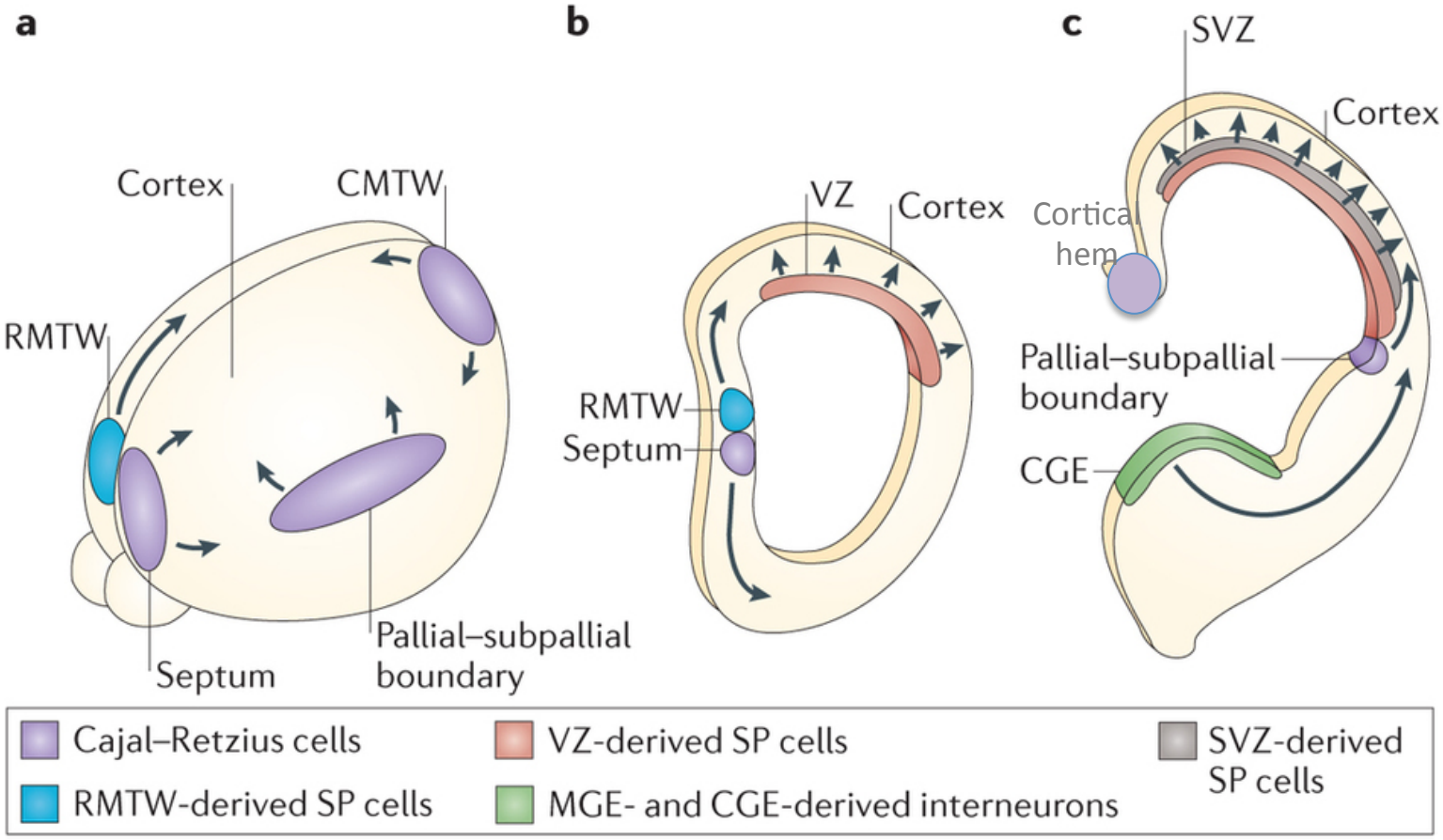
Reelin – extracellular matrix glycoprotein - plays also a role in synaptic plasticity in the mature brain

Cajal-Retzius cells are responsible for the correct lamination of the neocortex through the secretion of a **large secreted glycoprotein** called **Reelin**



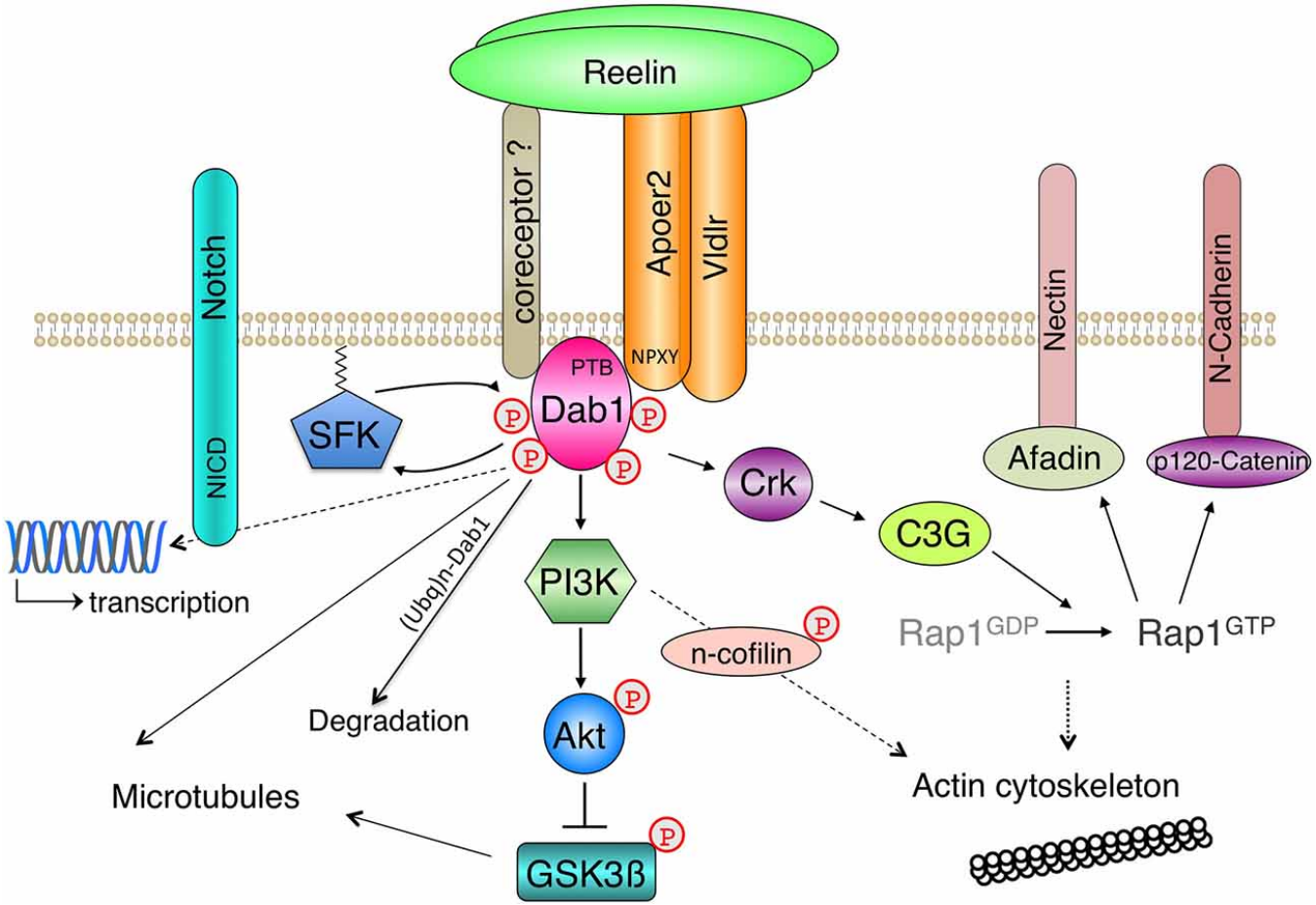
Multiple origin of CR cells

Cajal–Retzius (CR) cells primarily migrate into neocortical layer I from non-cortical locations (**caudomedial cortical hem**, the pallial-subpallial boudary and the septum)



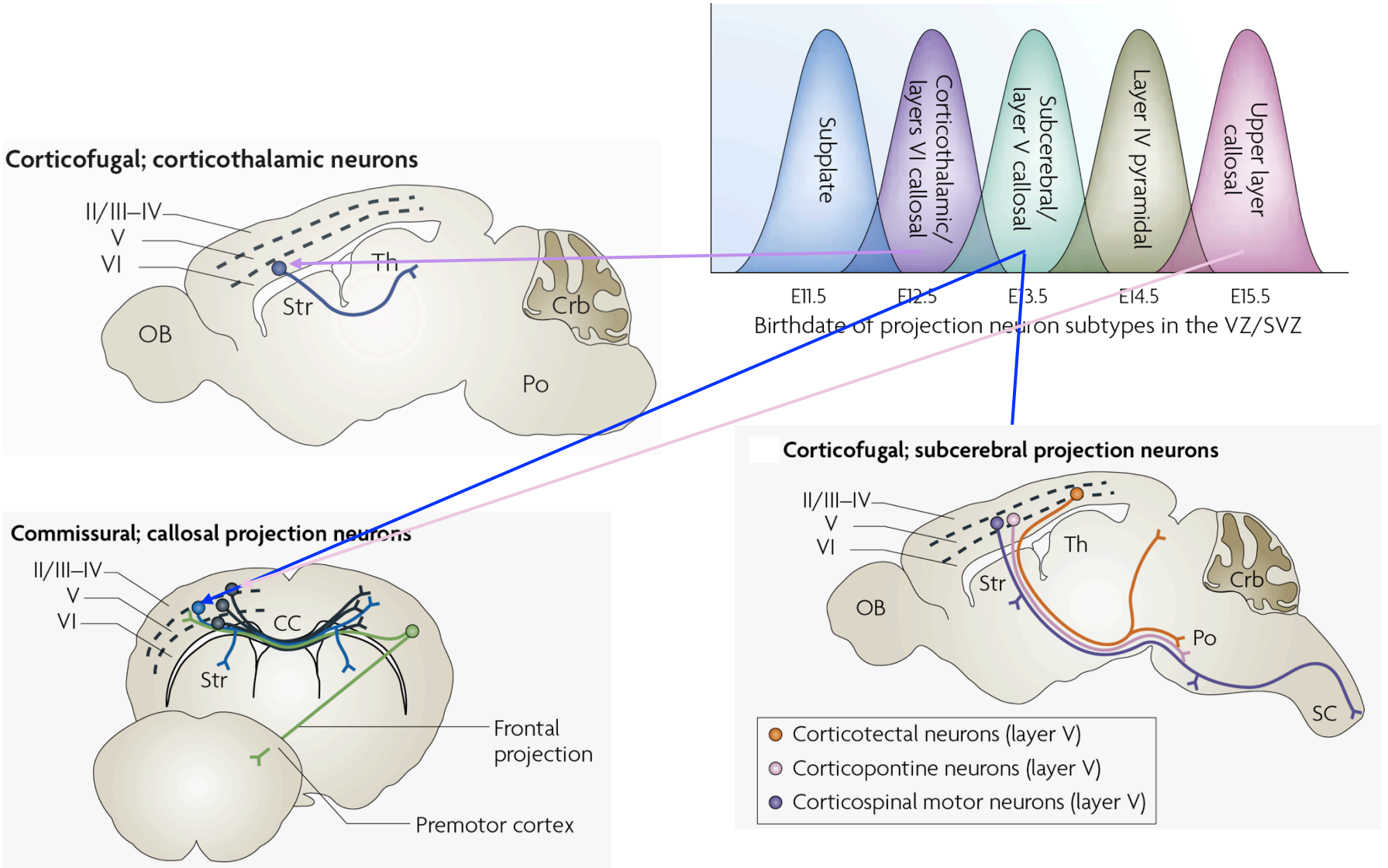
CMTW= caudo-medial telencephalic wall
RMTW=rostro-medial telencephalic wall

Nature Reviews | Neuroscience



Apolipoprotein E receptor-2 (Apoer2)
very low density-lipoprotein receptor(Vldlr)
Disabled-1(Dab1)

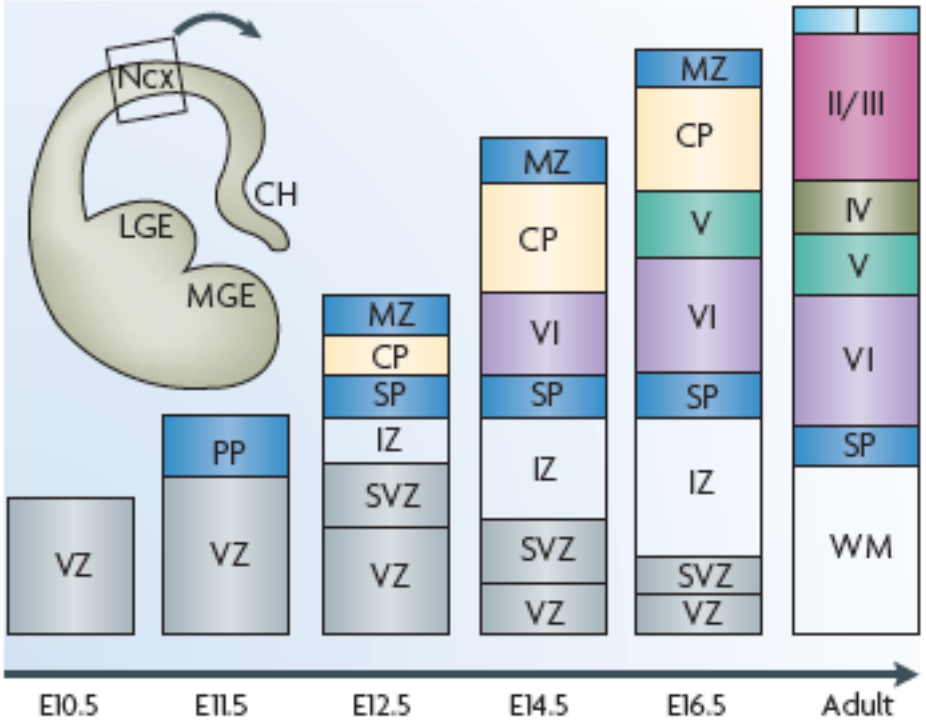
Sequential Specification of Cortical Projection Neurons



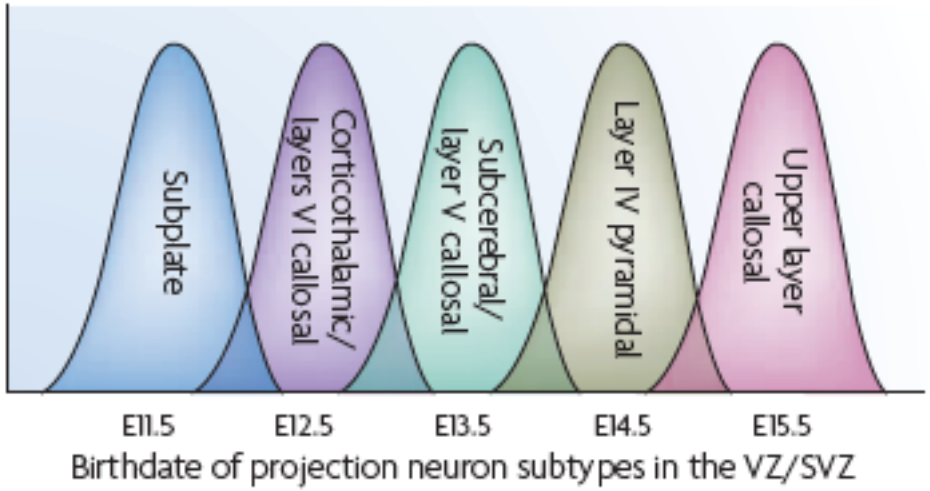
Molyneaux et al., Nature Rev. Neurosci., 2007

Time-dependent

Cortical progenitors generate different layers of neurons at distinct developmental stages



the birthdate of cortical projection neurons is intimately associated with laminar identity, axonal connectivity and physiological function



Birthdate of projection neuron subtypes in the VZ/SVZ

Different classes of projection neuron are born in overlapping temporal waves.

How is pyramidal neuron's temporal-identity specified?

What is the relationship between progenitor and neuronal diversity?

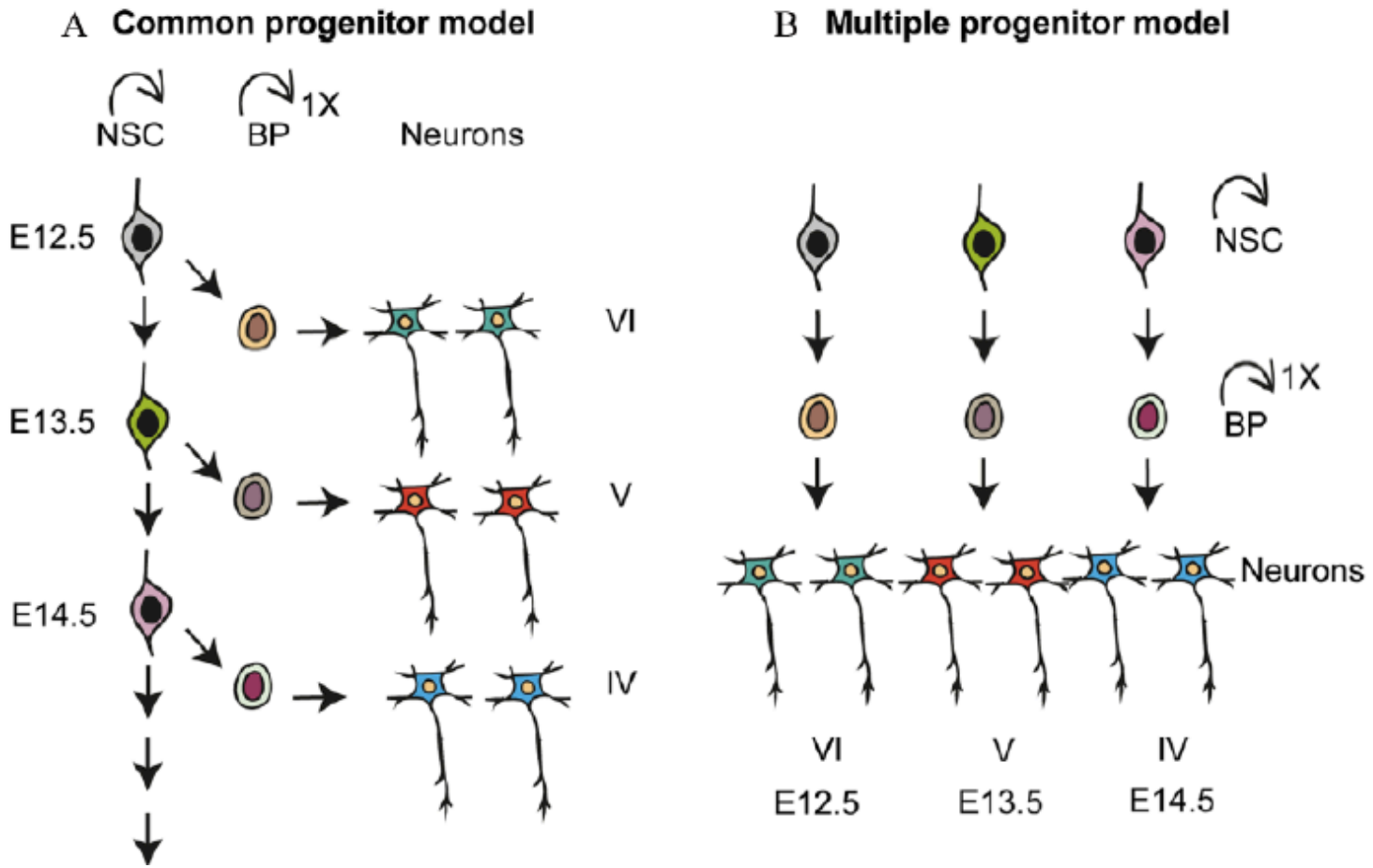
- Progressive temporal restriction of progenitor fate

OR

- Existence of classes of progenitors pre-committed to generate specific neuronal subtypes

?

What is the relationship between progenitor and neuronal diversity?



*A **single type of multipotent NSC** sequentially gives rise to different neuron cell subtypes*

*Coexistence of **multiple NSC types** predetermined to generate specific neuron subtypes*

Mukhtar and Verdon 2018

The Common Progenitor model…

ferret

Cell Cycle Dependence of Laminar Determination in Developing Neocortex

Science 1991

SUSAN K. MCCONNELL* AND CHRISTINE E. KAZNOWSKI

The neocortex is patterned in layers of neurons that are generated in an orderly sequence during development. This correlation between cell birthday and laminar fate prompted an examination of how neuronal phenotypes are determined in the developing cortex. At various times after labeling with [^{3}H]thymidine, embryonic progenitor cells were transplanted into older host brains. The laminar fate of transplanted neurons correlates with the position of their progenitors in the cell cycle at the time of transplantation. Daughters of cells transplanted in S-phase migrate to layer 2/3, as do host neurons. Progenitors transplanted later in the cell cycle, however, produce daughters that are committed to their normal, deep-layer fates. Thus, environmental factors are important determinants of laminar fate, but embryonic progenitors undergo cyclical changes in their ability to respond to such cues.

Neuron, Vol. 17, 55–61, July, 1996, Copyright ©1996 by Cell Press

NSCs become restricted over time during development

Restriction of Late Cerebral Cortical Progenitors to an Upper-Layer Fate

Gretchen D. Frantz and Susan K. McConnell
Department of Biological Sciences
Stanford University
Stanford, California 94305

Summary

Early in development, neural progenitors in cerebral cortex normally produce neurons of several layers during successive cell divisions. The laminar fate of their daughters depends on environmental cues encountered just before mitosis. At the close of neurogenesis, however, cortical progenitors normally produce neurons destined only for the upper layers. To assess the developmental potential of these cells, upper-layer progenitors were transplanted into the cerebral cortex of younger hosts, in which deep-layer neurons were being generated. These studies reveal that late cortical progenitors are not competent to generate deep-layer neurons and are instead restricted to producing the upper layers.

cells normally produce a smaller subset of laminar phenotypes, but these cells also display molecular differences compared with ventricular cells early in development: late progenitor cells express only low levels of the homeodomain gene *Otx1*, while early progenitors express *Otx1* at high levels (Frantz et al., 1994). Together, these findings raise the possibility that the developmental potential of late progenitors may be distinct from that of their earlier counterparts. Should this prove to be the case, the mechanisms that specify the laminar fates of cortical neurons might change over time during development. Although there are now many examples of cell–cell interactions that induce the production of specific cell types in the vertebrate nervous system (e.g., Shah et al., 1994; Roelink et al., 1995; Cepko et al., 1996), the possible contribution of lineage-based mechanisms is less well characterized. Here, we explore the mechanisms of laminar determination by late cortical progenitor cells and find that these cells are committed to the production of upper-layer neurons in a manner that is independent of environmental cues and heritable through successive cell divisions.

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DEV9710

Progressive restriction in fate potential by neural progenitors during cerebral cortical development

Aparna R. Desai and Susan K. McConnell*

Department of Biological Sciences, Stanford University, Stanford, California 94305, USA
*Author for correspondence (e-mail: suemcc@stanford.edu)

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SUMMARY

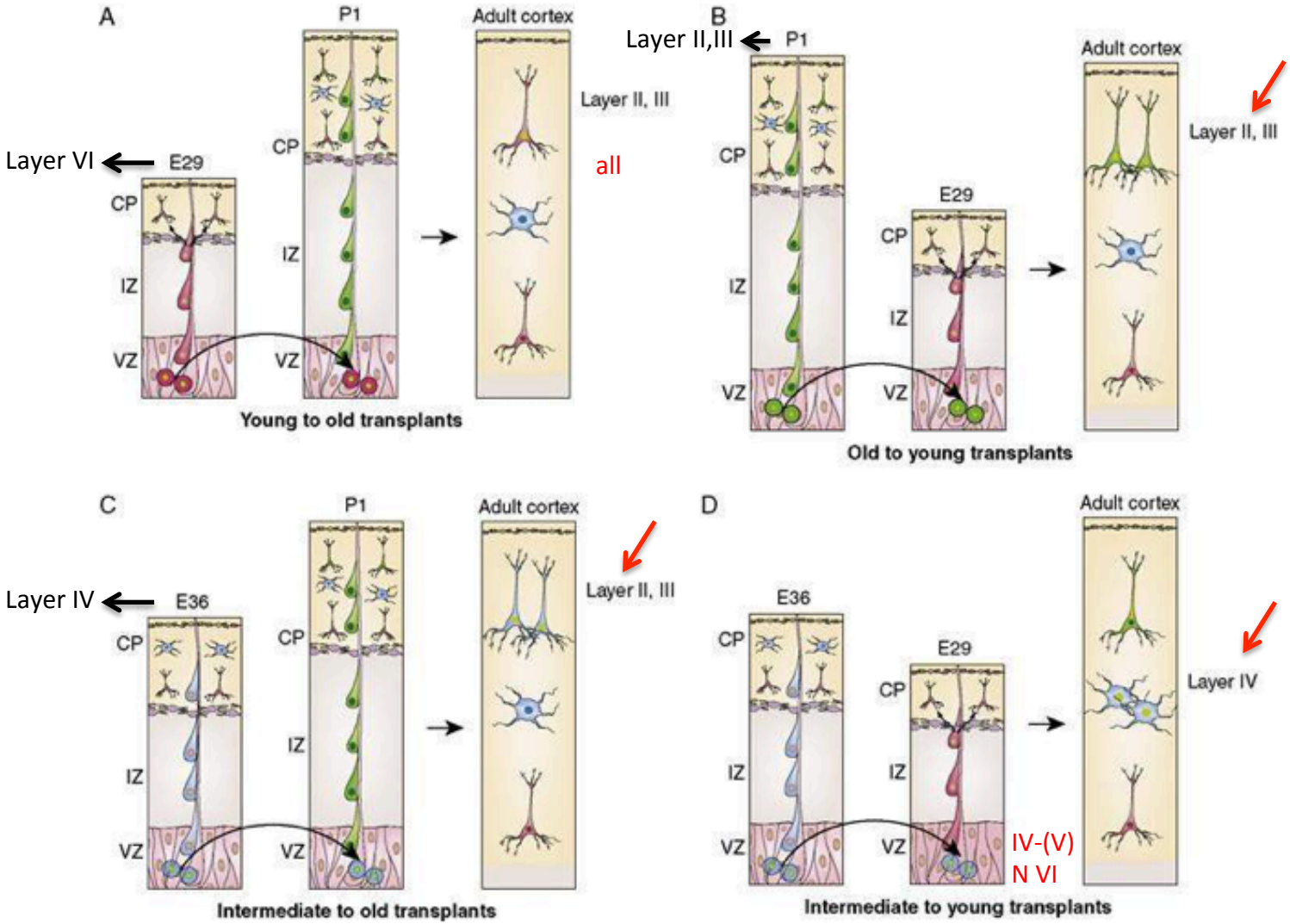
During early stages of cerebral cortical development, progenitor cells in the ventricular zone are multipotent, producing neurons of many layers over successive cell divisions. The laminar fate of their progeny depends on environmental cues to which the cells respond prior to mitosis. By the end of neurogenesis, however, progenitors are lineally committed to producing upper-layer neurons. Here we assess the laminar fate potential of progenitors at a middle stage of cortical development. The progenitors of layer 4 neurons were first transplanted into older brains in which layer 2/3 was being generated. The transplanted neurons adopted a laminar fate appropriate for the new environment (layer 2/3), revealing that layer 4 progenitors are multipotent. Mid-stage progenitors were then transplanted into a younger environment, in which layer 6 neurons were being generated. The transplanted neurons bypassed layer 6, revealing that layer 4 progenitors have a restricted fate potential and are incompetent to respond to environmental cues that trigger layer 6 production. Instead, the transplanted cells migrated to layer 4, the position typical of their origin, and also to layer 5, a position appropriate for neither the host nor the donor environment. Because layer 5 neurogenesis is complete by the stage that progenitors were removed for transplantation, restrictions in laminar fate potential must lag behind the final production of a cortical layer. These results suggest that a combination of intrinsic and environmental cues controls the competence of cortical progenitor cells to produce neurons of different layers.

Key words: Fate determination, Neurogenesis, Lineage, Cerebral cortex, Migration, Ferret

Heterochronic transplantation

Early stage progenitors can generate early and late neuronal subtypes
Late stage progenitors → restricted potential (loose potential to generate deep neurons)

Ferrets
Gestation is around 6 weeks (38 to 44 days)



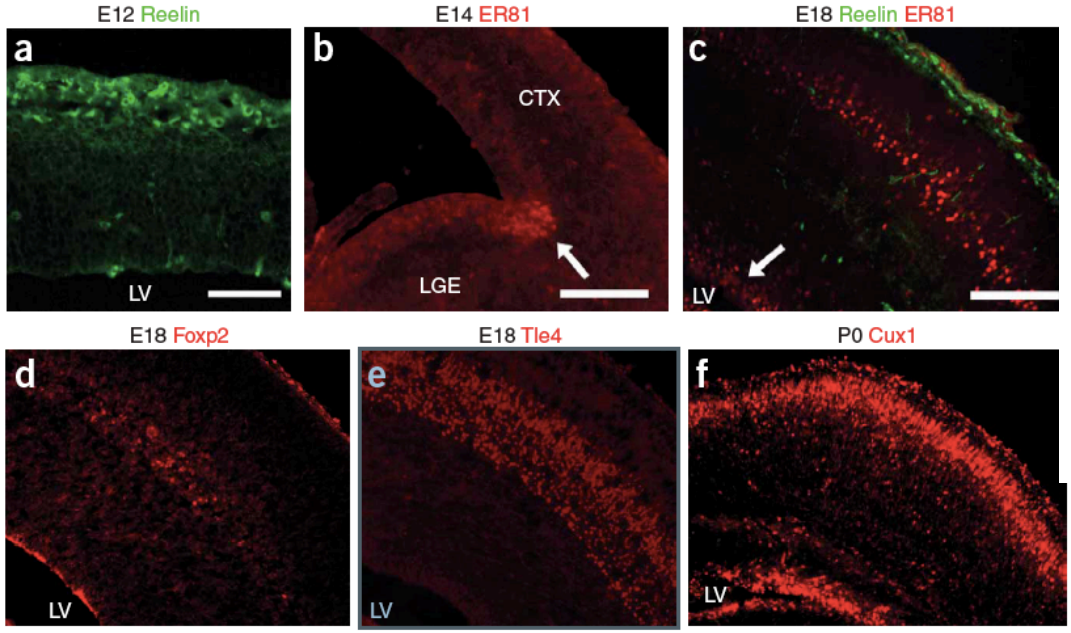
Nature Neurosci 2006

The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells

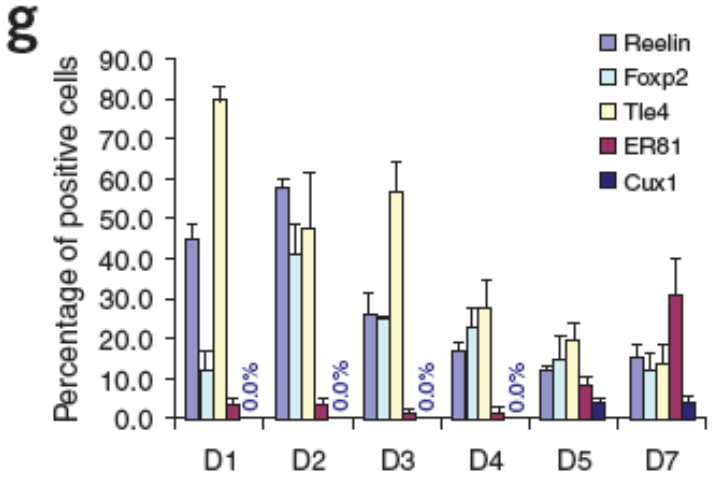
Qin Shen[1], Yue Wang[1], John T Dimos[2], Christopher A Fasano[1], Timothy N Phoenix[1], Ihor R Lemischka[2], Natalia B Ivanova[2], Stefano Stifani[3], Edward E Morrisey[4] & Sally Temple[1]

In the developing cerebral cortex, neurons are born on a predictable schedule. Here we show in mice that the essential timing mechanism is programmed within individual progenitor cells, and its expression depends solely on cell-intrinsic and environmental factors generated within the clonal lineage. Multipotent progenitor cells undergo repeated asymmetric divisions, sequentially generating neurons in their normal *in vivo* order: first preplate cells, including Cajal-Retzius neurons, then deep and finally superficial cortical plate neurons. As each cortical layer arises, stem cells and neuroblasts become restricted from generating earlier-born neuron types. Growth as neurospheres or in co-culture with younger cells did not restore their plasticity. Using short-hairpin RNA (shRNA) to reduce *Foxg1* expression reset the timing of mid- but not late-gestation progenitors, allowing them to remake preplate neurons and then cortical-plate neurons. Our data demonstrate that neural stem cells change neuropotency during development and have a window of plasticity when restrictions can be reversed.

Timing of appearance of diverse cortical neurons



E 10.5 cortex progenitors → in vitro



The appearance of layer-specific neurons in culture follows the same order as in vivo development

In vivo studies

→ heterochronic transplantation = evidences of **progressive restriction** of progenitor potential (Mc Connel group) *(early progenitors are multipotent –late progenitors are not)*

→ Retroviral lineage fate-mapping (Luskin et al., Neuron 1988, Walsh et al., Science 1988) support progressive fate restriction in vivo

In vitro

→multipotent progenitors in culture give rise to deep layer neurons first and upper layer neurons later

→murine ES cells differentiating into cortical PN-like cells show a temporal pattern of sequential neuronal generation

all projection neurons may be generated from the **same multipotent progenitor** →fate distinction are mostly temporally controlled

→cortical progenitor subtypes are sequentially determined by birthdate through progressive lineage restriction of a common RGC

The Multiple Progenitor model…

Science 2012

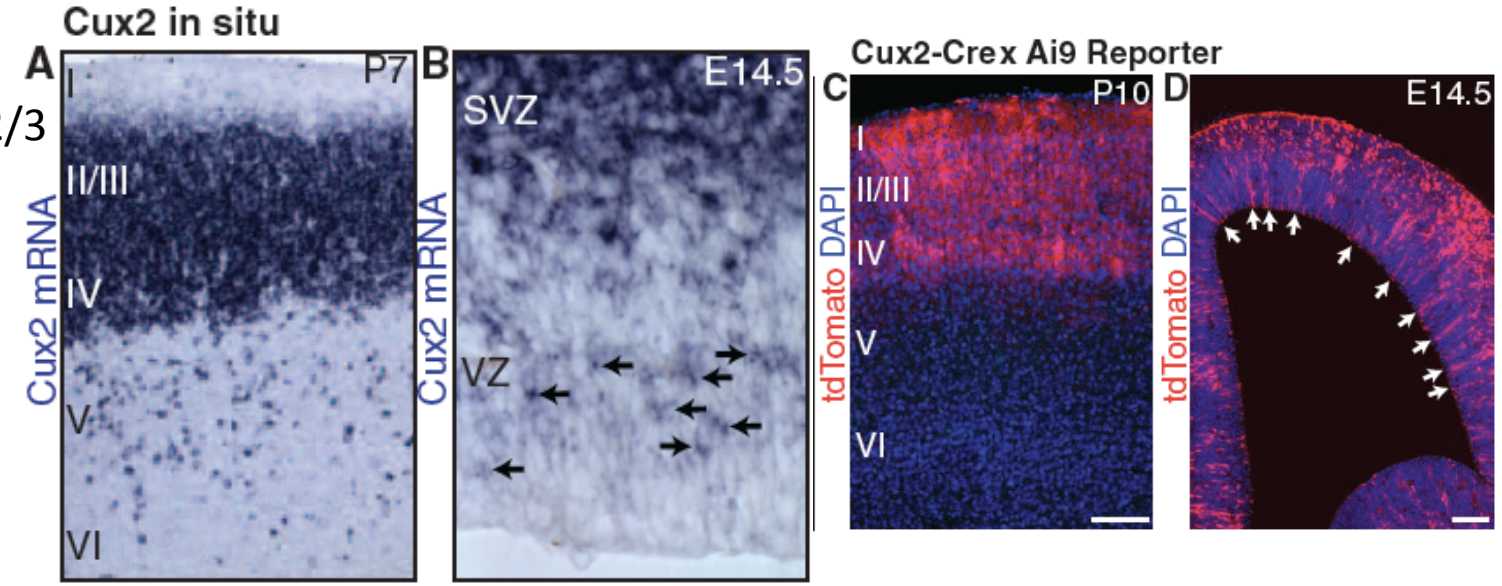
Cux2 RGC lineage is **intrinsically specified** to generate **callosal upper-layer neurons**, independently of niche and birthdate

Fate-Restricted Neural Progenitors in the Mammalian Cerebral Cortex

Santos J. Franco, Cristina Gil-Sanz,* Isabel Martinez-Garay,*† Ana Espinosa, Sarah R. Harkins-Perry, Cynthia Ramos, Ulrich Müller‡

During development of the mammalian cerebral cortex, radial glial cells (RGCs) generate layer-specific subtypes of excitatory neurons in a defined temporal sequence, in which lower-layer neurons are formed before upper-layer neurons. It has been proposed that neuronal subtype fate is determined by birthdate through progressive restriction of the neurogenic potential of a common RGC progenitor. Here, we demonstrate that the murine cerebral cortex contains RGC sublineages with distinct fate potentials. Using in vivo genetic fate mapping and in vitro clonal analysis, we identified an RGC lineage that is intrinsically specified to generate only upper-layer neurons, independently of niche and birthdate. Because upper cortical layers were expanded during primate evolution, amplification of this RGC pool may have facilitated human brain evolution.

Callosal PN L2/3



Neuron
Report

2013

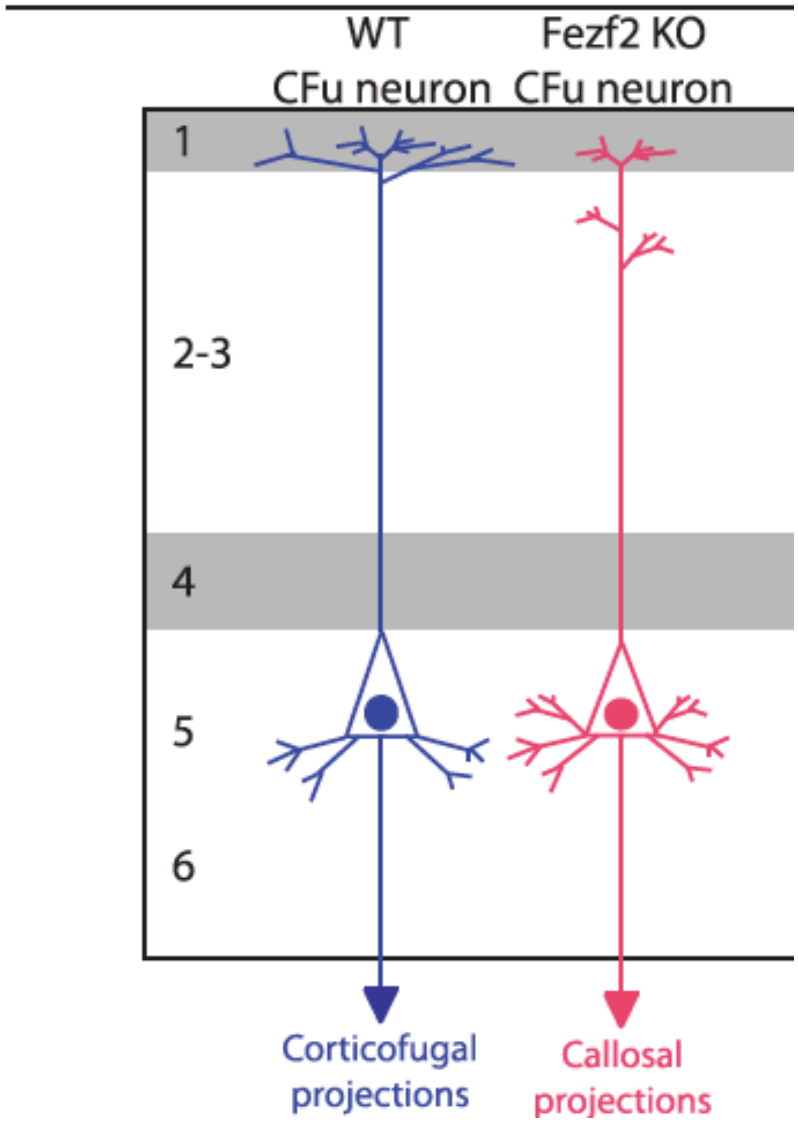
Fezf2 Expression Identifies a Multipotent Progenitor for Neocortical Projection Neurons, Astrocytes, and Oligodendrocytes

Chao Guo,[1,3] Matthew J. Eckler,[1,3] William L. McKenna,[1] Gabriel L. McKinsey,[2] John L.R. Rubenstein,[2] and Bin Chen[1,*]
[1]Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA 95064, USA
[2]Department of Psychiatry, Neuroscience Program, and the Nina Ireland Laboratory of Developmental Neurobiology, University of Ca
San Francisco, CA 94158, USA
[3]These authors contributed equally to this work
*Correspondence: bchen@ucsc.edu
http://dx.doi.org/10.1016/j.neuron.2013.09.037

SUMMARY

Progenitor cells in the cerebral cortex sequentially generate distinct classes of projection neurons. Recent work suggests the cortex may contain intrinsically fate-restricted progenitors marked by expression of Cux2. However, the heterogeneity of the neocortical ventricular zone as well as the contribution of lineage-restricted progenitors to the overall cortical neurogenic program remains unclear. Here, we utilize in vivo genetic fate mapping to demonstrate that Fezf2-expressing radial glial cells (RGCs) exist throughout cortical development and sequentially generate all major projection neuron subtypes and glia. Moreover, we show that the vast majority of CUX2$^+$ cells in the VZ and SVZ are migrating interneurons derived from the subcortical telencephalon. Examination of the embryonic cortical progenitor population demonstrates that Cux2$^+$ RGCs generate both deep- and upper-layer projection neurons. These results identify Fezf2$^+$ radial glial cells as a multipotent neocortical progenitor and suggest that the existence, and molecular identity, of laminar-fate-restricted RGCs awaits further investigation.

Fezf2 (forebrain embryonic zinc finger 2)
one of the master genes for the specification of cortifugal (subcerebral) neurons



Fezf2
→ is expressed in VZ progenitors during the generation of deep layer neurons (is progressively downregulated and disappears by the time upper layer neurons are produced)
→ its expression is maintained in postmitotic neurons of layer V and VI

In the absence of Fezf2 function in **Fezf2 KO** mice **the entire population of subcerebral projection neuron is absent**: cells in the V layer are there but there are no projections from the cerebral cortex to the spinal cord and brainstem

Nat Cell Biol. 2013 February ; 15(2): 214–221. doi:10.1038/ncb2660.

https://youtu.be/_QXUfQ5kgjE

Direct lineage reprogramming of postmitotic callosal neurons into corticofugal neurons in vivo

Caroline Rouaux[1,2] and **Paola Arlotta**[1,*]

[1]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA., Sherman Fairchild Building, 7 Divinity Avenue, Cambridge MA 02138

Abstract

Once programmed to acquire a specific identity and function, cells rarely change *in vivo*[1]. Neurons of the mammalian central nervous system (CNS) in particular are a classic example of a stable, terminally differentiated cell type. With the exception of the adult neurogenic niches, where a limited set of neuronal subtypes continue to be generated throughout life[2,3], CNS neurons are only born during embryonic and early postnatal development. Once generated, neurons become permanently postmitotic and do not change their identity for the life span of the organism. Here, we have investigated whether excitatory neurons of the neocortex can be instructed to directly reprogram their identity postmitotically from one subtype into another, *in vivo*. We show that embryonic and early postnatal callosal projection neurons (CPN) of layer II/III can be postmitotically lineage reprogrammed into layer V/VI corticofugal projection neurons (CFuPN) upon expression of the transcription factor *Fezf2*. Reprogrammed callosal neurons acquire molecular properties of corticofugal projection neurons and change their axonal connectivity from interhemispheric, intracortical projections to corticofugal projections directed below the cortex. The data indicate that during a window of postmitotic development neurons can change their identity, acquiring critical features of alternate neuronal lineages.

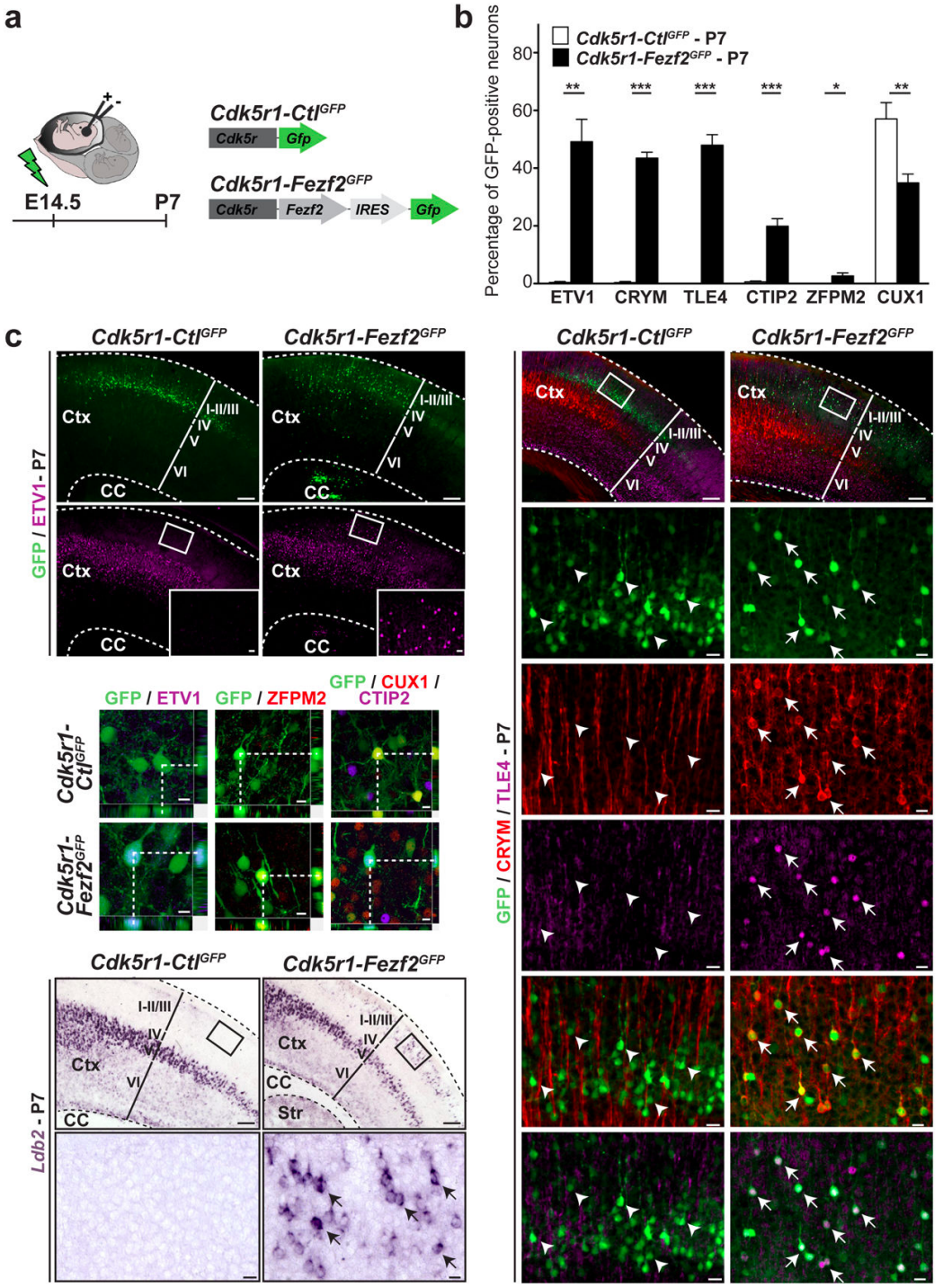
1. **Early postmitotic stages**

Cdk5r gene promoter to drive *Fezf2* expression in “young” postmitotic callosal projection neurons (CPN).

→ In utero electroporation (E14.5) of *Cdk5r-Fezf2eGFP* or control *Cdk5r-CtleGFP* expression vectors → analysis at E18.5, P7, P14 and P28.

Analysis of the molecular identity shows that young postmitotic CPN are able to acquire molecular features of CFuPN

in an early period of postmitotic life, CPN of layer II/III are able to reprogram their molecular identity, acquiring combinatorial expression of CFuPN proteins and repressing CPN proteins



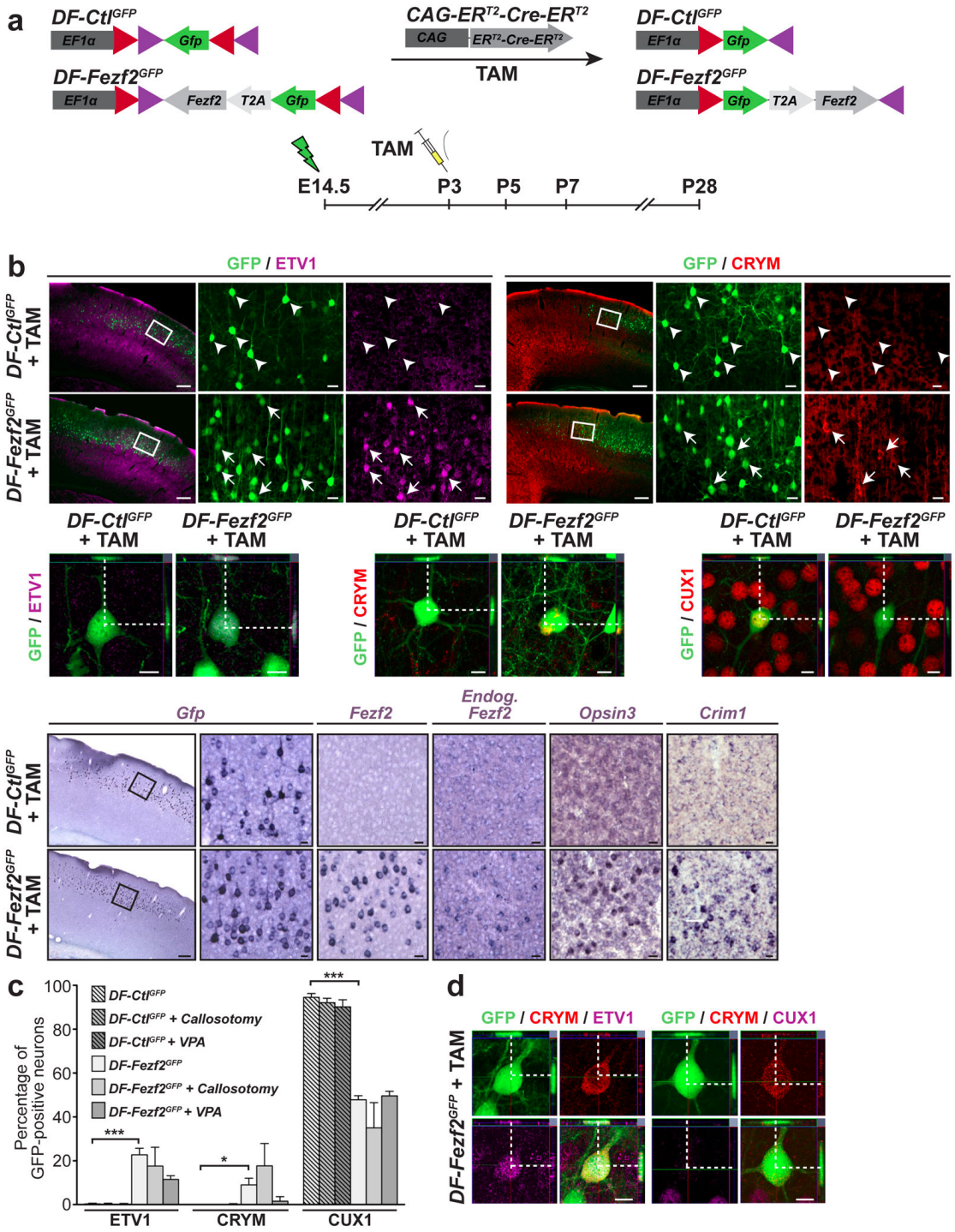
2. **Late postmitotic stages**

Conditional expression vector with the *Fezf2* expression cassette cloned in antisense orientation between two pairs of inverted LoxP sites (*DF-Fezf2eGFP*).

Electroporation of cortical progenitors at E14.5 with *DF-Fezf2eGFP* or control *DF-CtleGFP* together with a 4-hydroxytamoxifen (TAM)-inducible form of *Cre* recombinase.

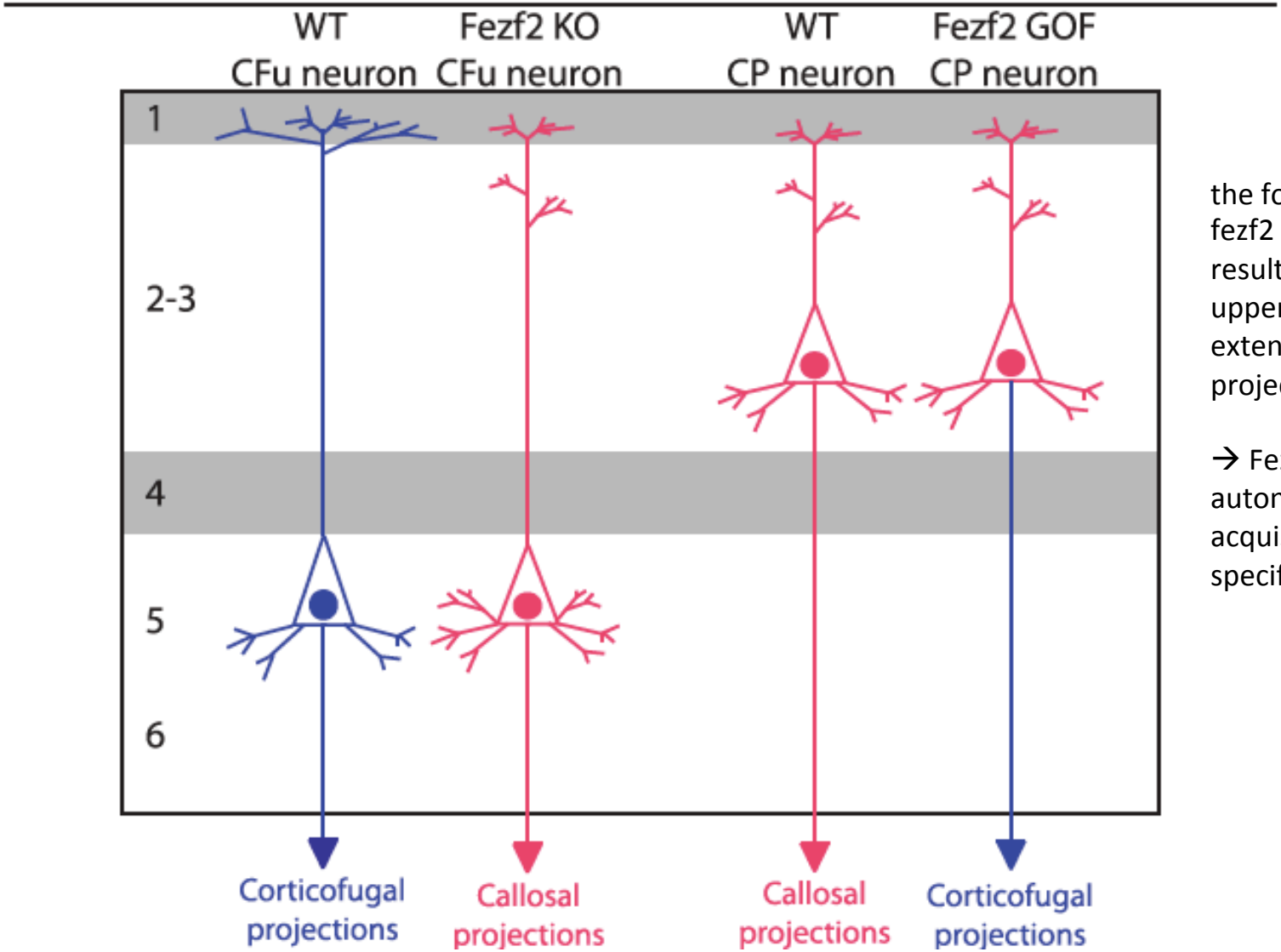
Recombination was induced by injections of TAM at either **E17.5, P3 or P21**

CPN that began to express *Fezf2* **at E17.5** and **P3** are still able to reprogram their molecular identity and expressed several CFuPN genes.



Fezf2 (forebrain embryonic zinc finger 2)
one of the master genes for the specification of cortifugal neurons

Fate switch



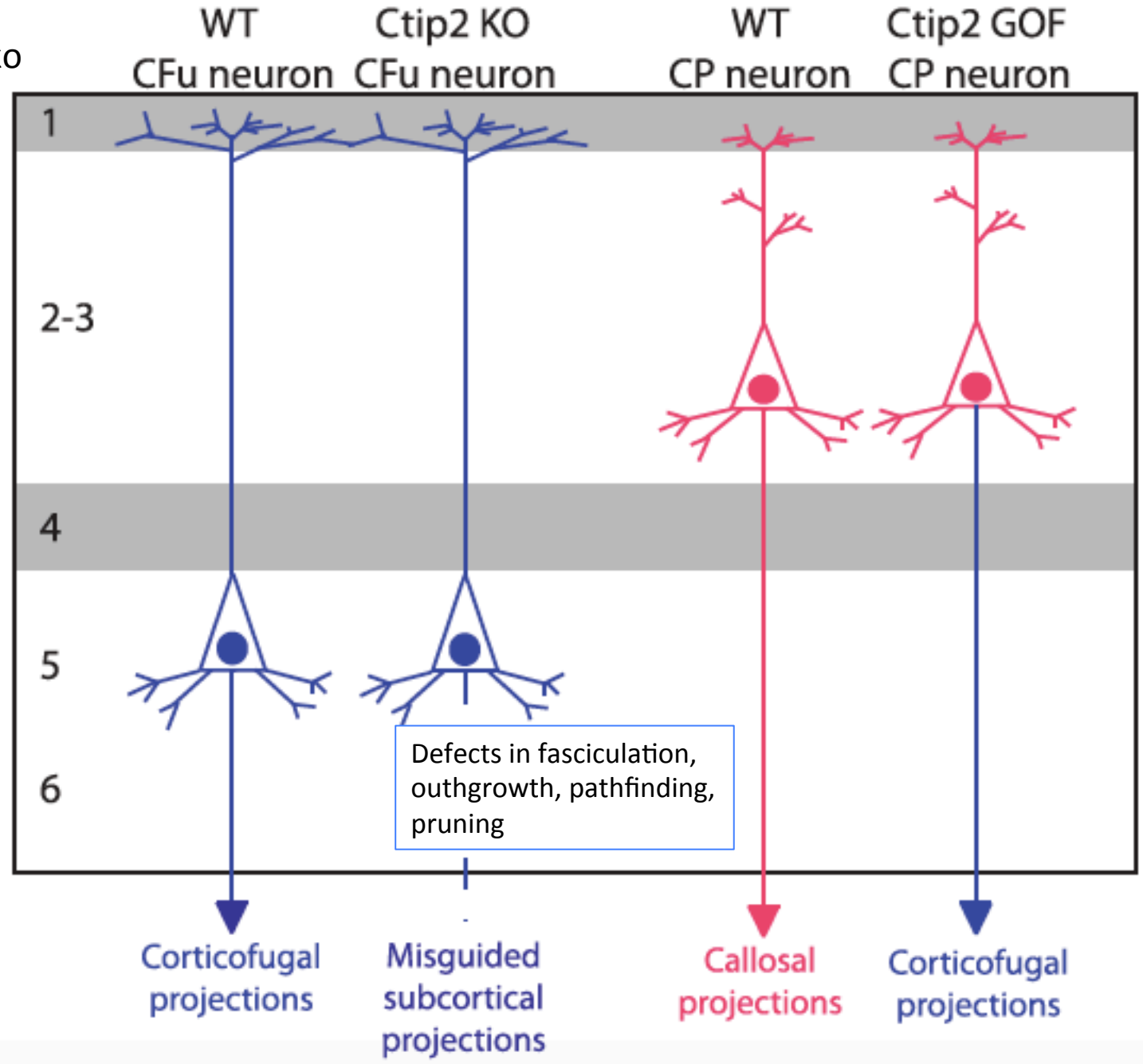
the forced expression of fezf2 in E17/P3 progenitors results in the generation of upper layer neurons that extend subcerebral axonal projections

→ Fezf2 alone can cell-autonomously instruct the acquisition of corticofugal specific features

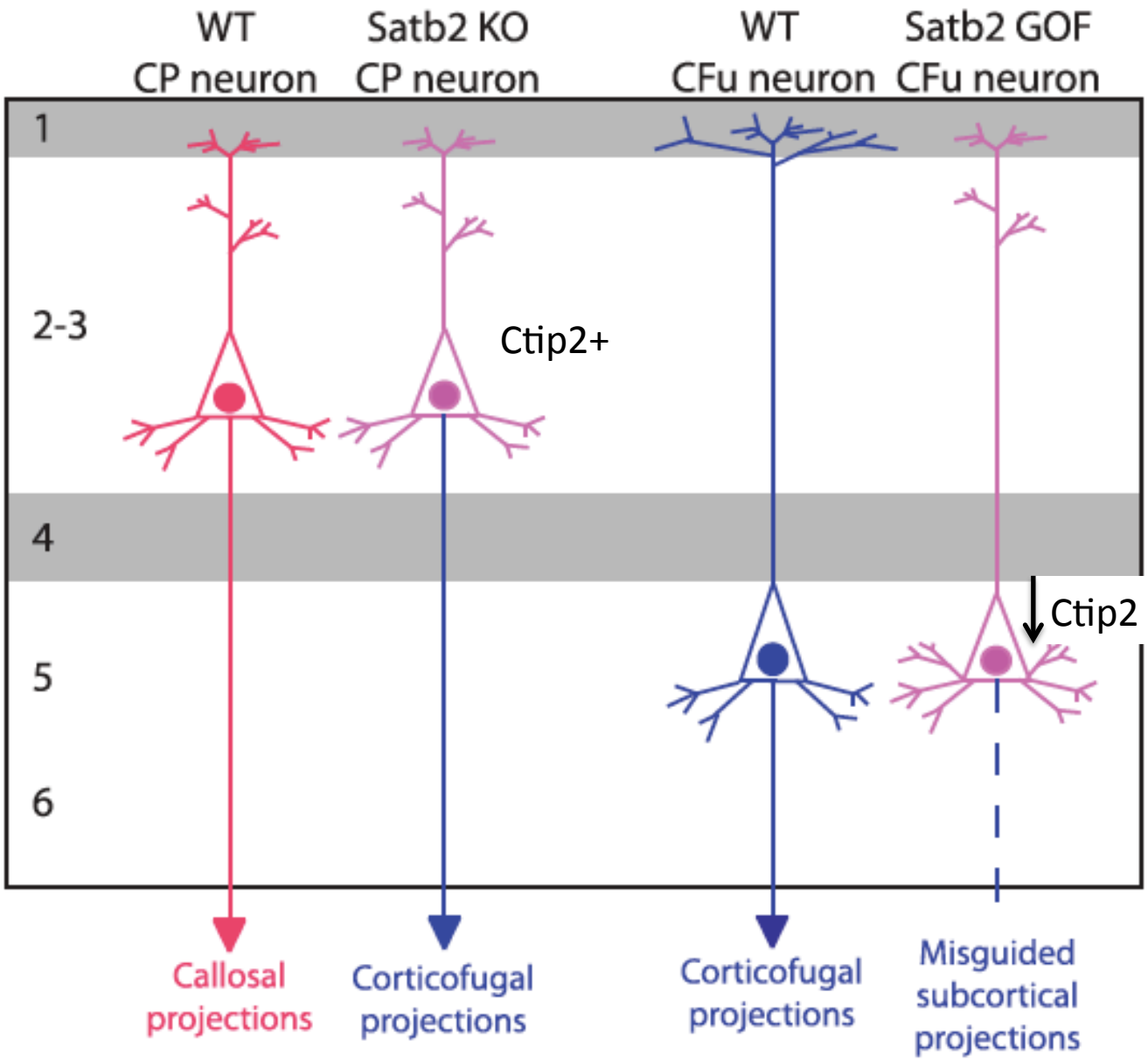
CTIP2 is downstream of Fetzf2

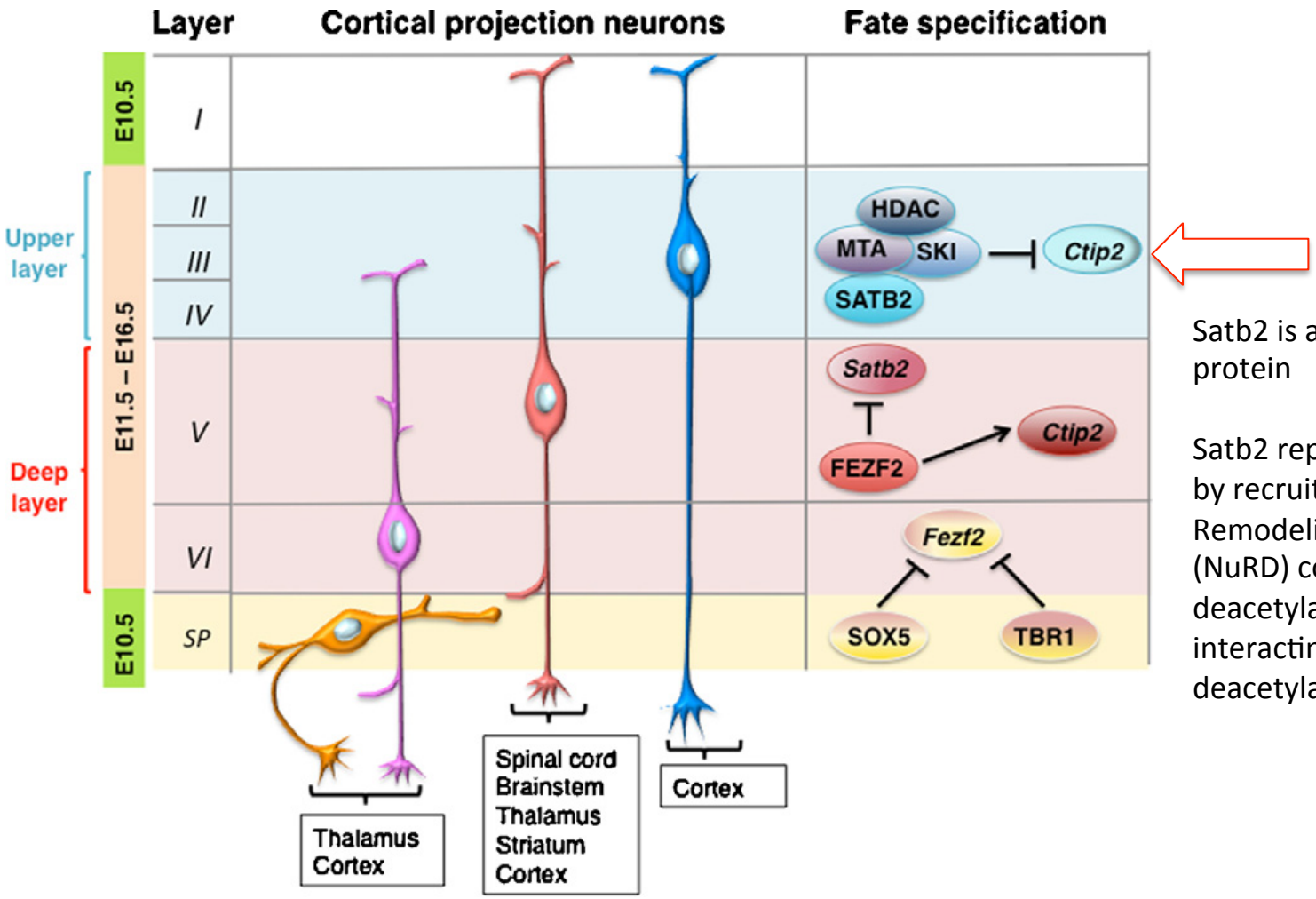
↓- In Fetzf2ko

(phenotype recovery of Fetzf2ko by CTIP2 GOF)



Satb2 and upper-layer neurons/callosal neurons specification





Satb2 is a chromatin remodeling protein

Satb2 represses Ctip2 expression by recruiting the Nucleosome Remodeling and Deacetylase (NuRD) complex, which in turn deacetylates the Ctip2 locus by interacting with the histone deacetylase 1 (Hdac1)

Mitotic vs Postmitotic

→TFs expressed **postmitotically** are important in controlling the generation of specific pyramidal neuron subtypes

→early laminar specification of neocortical neurons can be reprogrammed postmitotically (*Fezf2 Rouaux & Arlotta, 2013*)

→ reciprocal regulation between postmitotically expressed TFs to obtain progressive refinement of neural subtype identity during cortical development

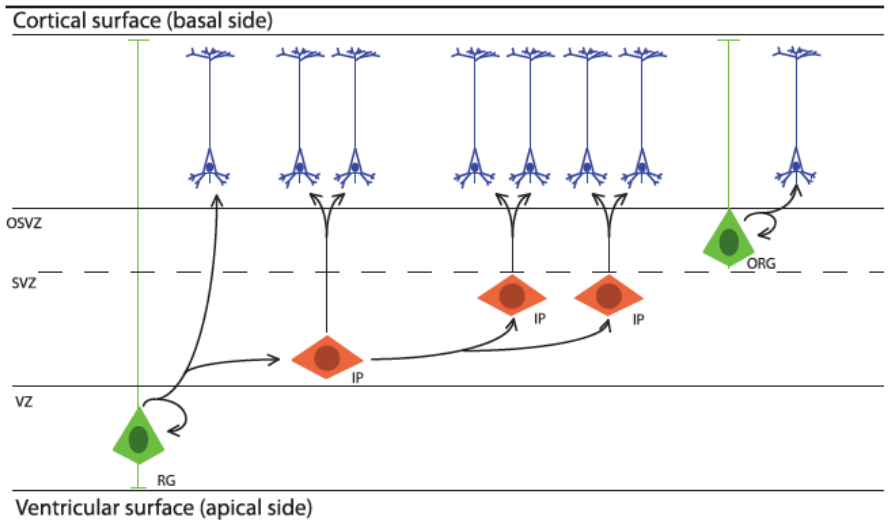
6142 • The Journal of Neuroscience, April 15, 2015 • 35(15):6142– 6152

A further level of complexity…..

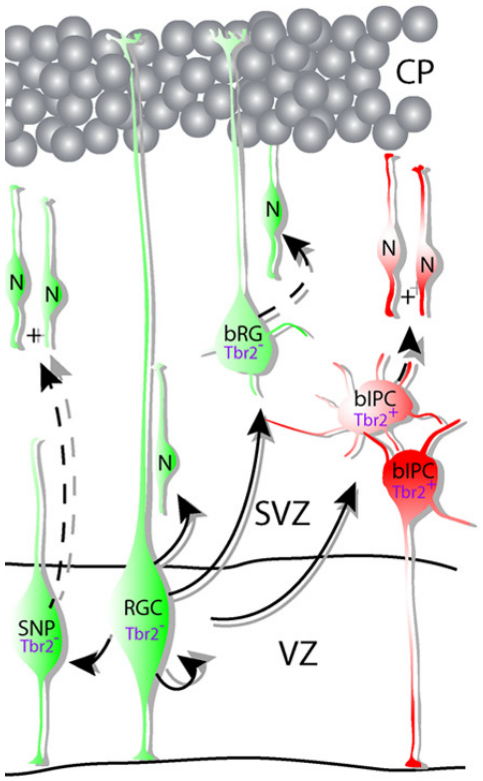
Development/Plasticity/Repair

Neural Precursor Lineages Specify Distinct Neocortical Pyramidal Neuron Types

Here we use a novel genetic fate mapping technique to simultaneously track multiple precursor streams in the developing mouse brain and show that layer 2 and 3 pyramidal neurons exhibit distinctive electrophysiological and structural properties depending upon their precursor cell type of origin. These data indicate that individual precursor subclasses synchronously produce functionally different neurons, even within the same lamina, and identify a primary mechanism leading to cortical neuronal diversity.



Do IPCs merely expand neuronal production or instead make distinct types of neurons?



The four major classes of neocortical precursors:
distinction based on differential TF expression

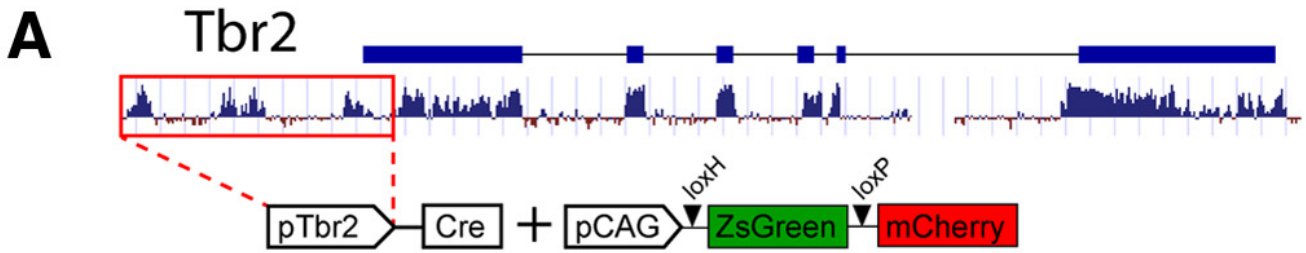
1-RGCs (Pax6+;Tbr2-)

2-aIPC (SNP) (Pax6+;Tbr2-)

3-bIPC (Tbr2+)

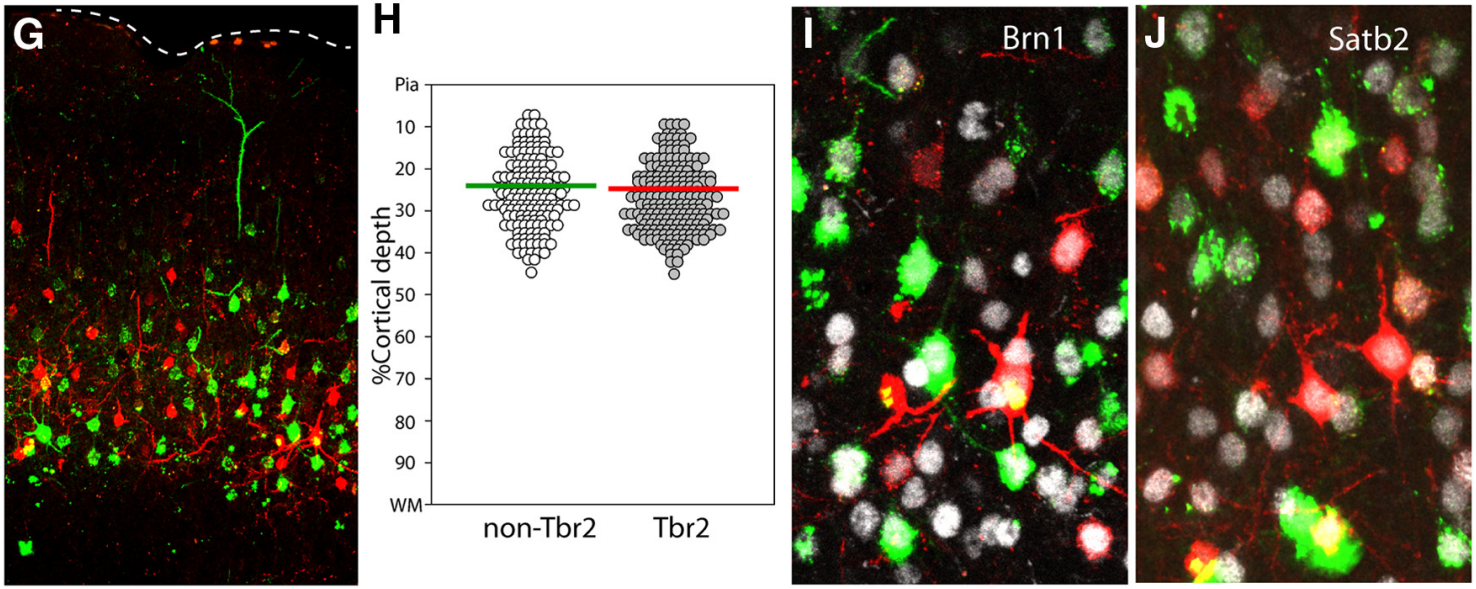
4-bRGC (Pax6+;Tbr2-)

In utero electroporation (**E14.5**):
-Tbr2 regulatory sequence-Cre
-dual fluorescent report plasmid



Analysis at P21

Tbr2+(red) and Tbr-(green) neurons are located in layer 2/3

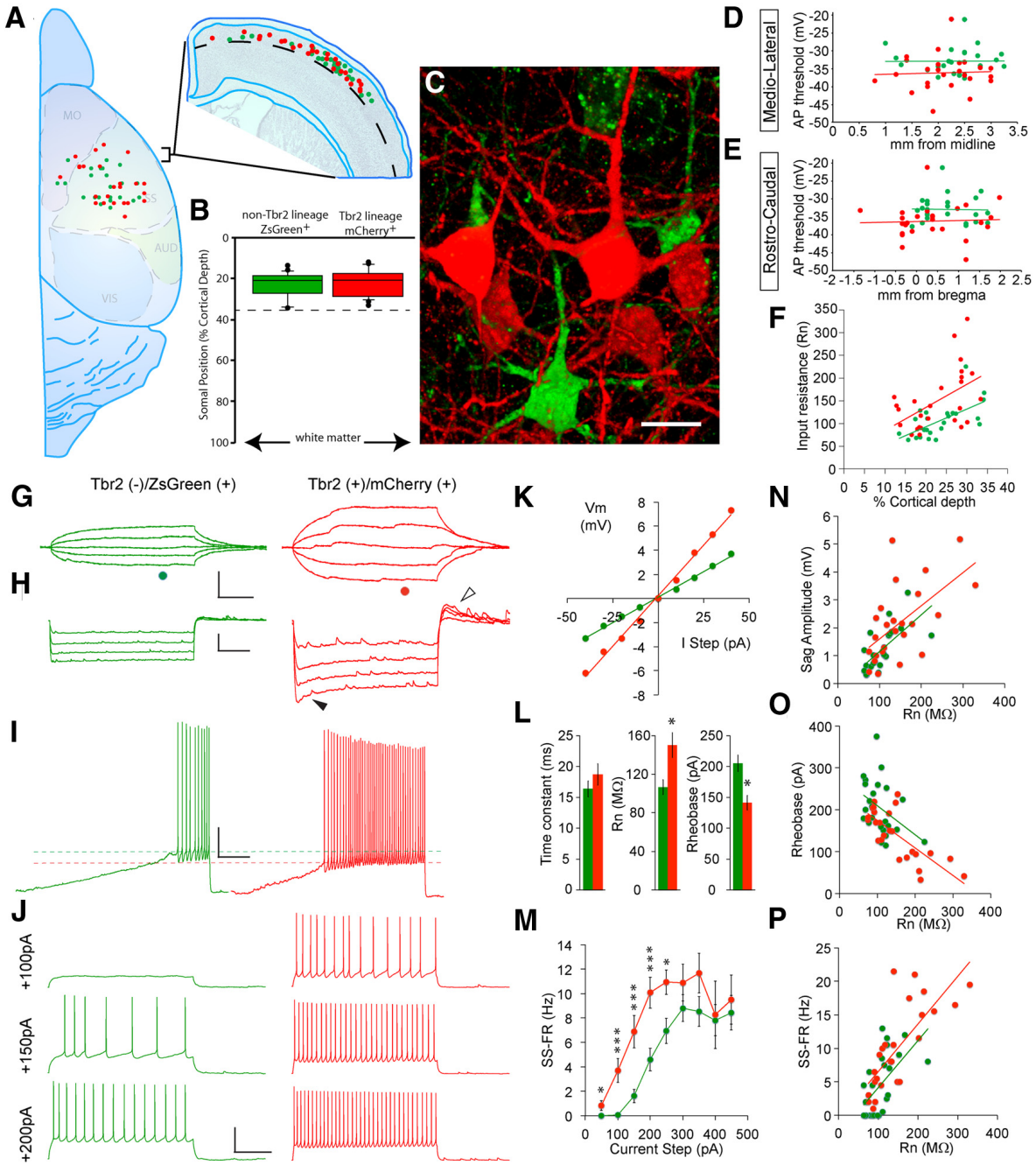


→ Separately fate mapped neuronal precursors simultaneously generate pyramidal neuron descendant fated to the supragranular layers

BUT

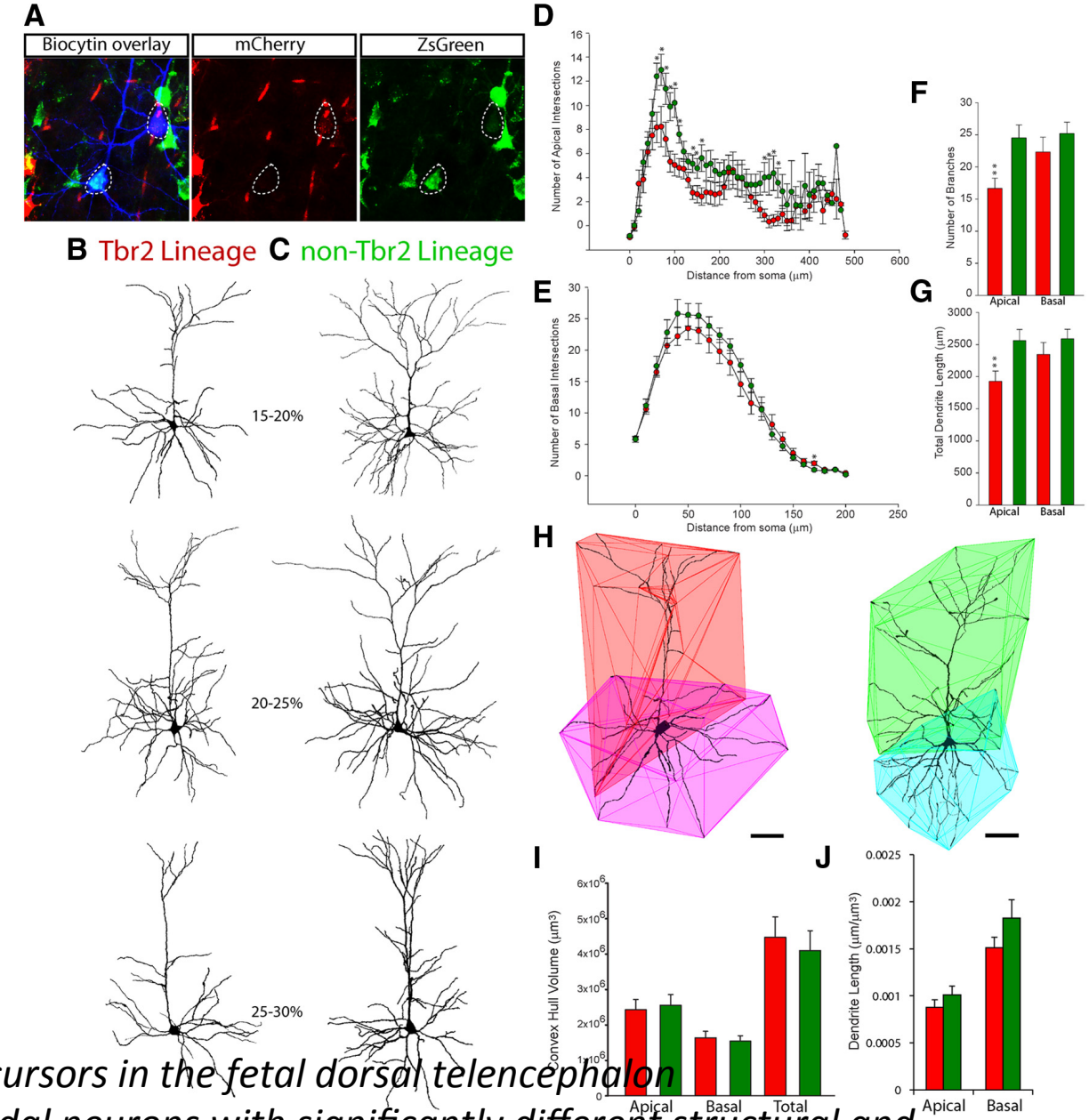
-different functional electrophysiological properties

higher excitability of Tbr2+ neurons



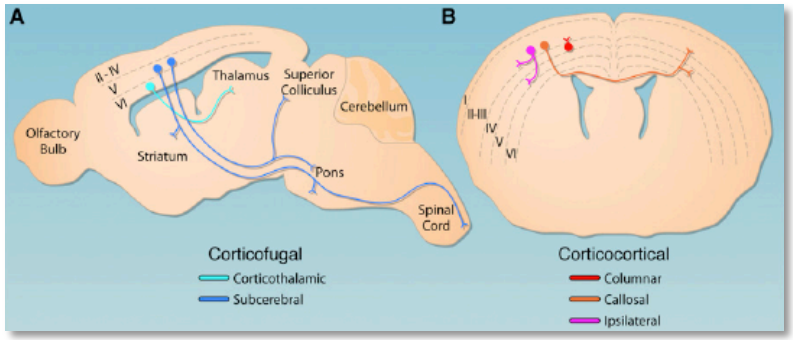
AND

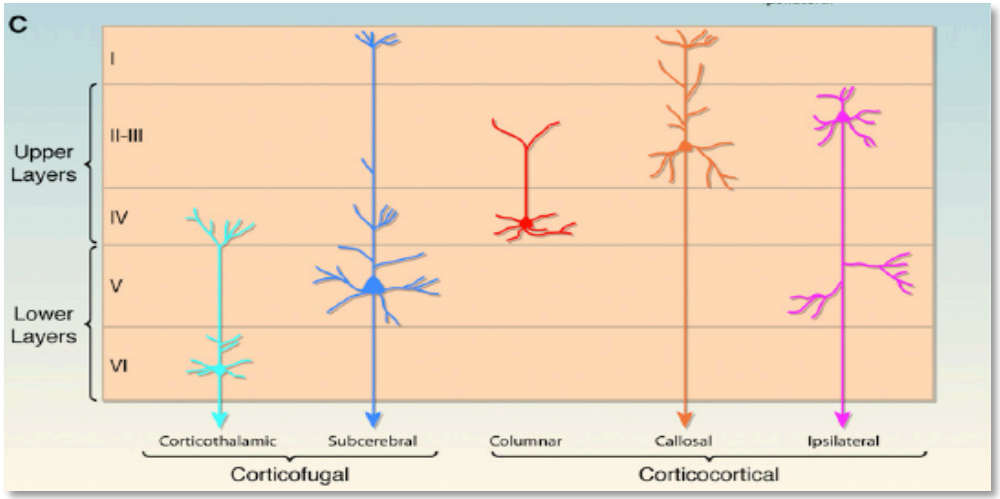
-different neuronal morphology



Multiple lineages of neural precursors in the fetal dorsal telencephalon simultaneously produce pyramidal neurons with significantly different structural and functional properties even when they are destined to reside within the same neocortical

Key determinants of neuronal specification



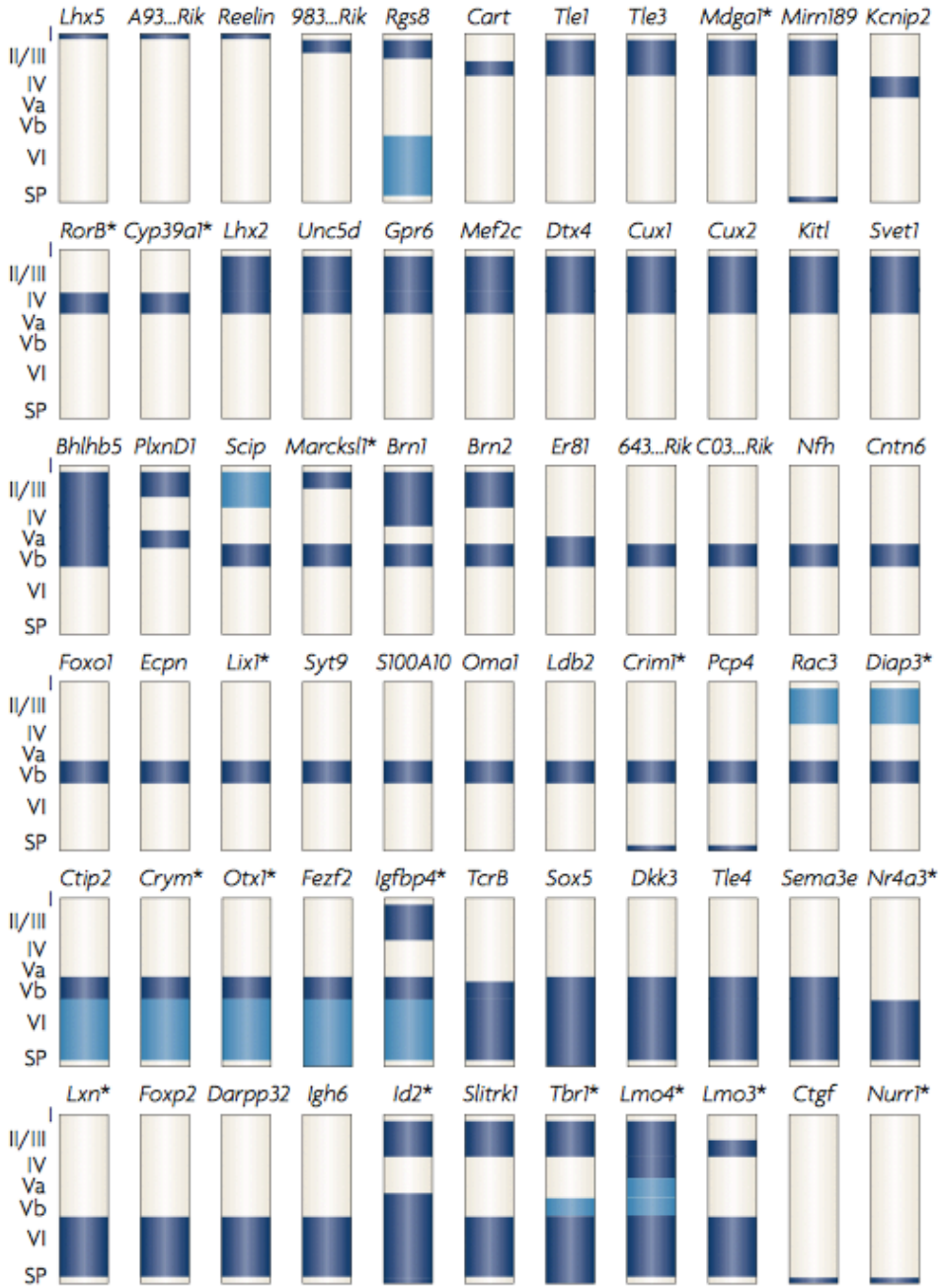


Fezf2 Ctip2 Sox5 Tbr1 RORB Cux1 Satb2 Lmo4 Bhlhb5

What is **the role of individual genes** in controlling the specification and development of distinct projection neuron subtypes?

Modified from Gaspard & Vanderhaeghen, 2011

Lamina and subtype-specific genes in the mouse neocortex



→ The combinatorial code is temporally dynamic (eg. *Ctip2)*

→ Transcript expression does not always reflect protein distribution *(e.g Ctip2 mRNA in progenitors + postmitotic; protein only postmitotic)*

→ Each neuronal population is heterogeneous (combinatorial code)

*genes which laminar or subtype expression vary by area within the neocortex

Molyneaux et al., 2007

DeCoN: Genome-wide Analysis of In Vivo Transcriptional Dynamics during Pyramidal Neuron Fate Selection in Neocortex

Bradley J. Molyneaux,[1,5,6] Loyal A. Goff,[1,2,3,5,7] Andrea C. Brettler,[1] Hsu-Hsin Chen,[1] Juliana R. Brown,[1] Siniša Hrvatin,[1] John L. Rinn,[1,2,4,*] and Paola Arlotta[1,2,*]

Molyneaux et al., 2015, Neuron 85, 275–288

+ VIDEO + web site

DeCoN | Marker Discovery | Clusters | All Genes | Supplement | About | Gene Set | Gene name or Locus | Search

DeCoN
Developing Cortical Neuron Transcriptome Resource

The Developing Cortical Neuron Transcriptome Resource

Neuronal development requires a complex choreography of transcriptional decisions to obtain a specific cellular identity. Yet the poor accessibility of individual neuronal classes has made direct measurements of gene expression difficult. We have combined nuclear-antigen targeting FACS sorting with high-resolution massively parallel RNA sequencing to provide the first resource of deep-transcriptomic data in individual sorted neuron populations. We provide here, an interactive space to explore the numerous aspects of these data including spatial and temporal usage of alternative mRNA isoforms and promoters, to a host of mRNA and long noncoding RNA (lncRNA) genes newly implicated in neuronal cell fate specification. This database enables integrated, multidimensional data mining and provides a powerful resource to generate insights into the transcriptional regulation underlying neuronal diversity in the developing cortex.

DeCoN provides an exploratory interface to deep transcriptional profiling data of three clinically relevant subclasses of mouse cortical projection neurons:

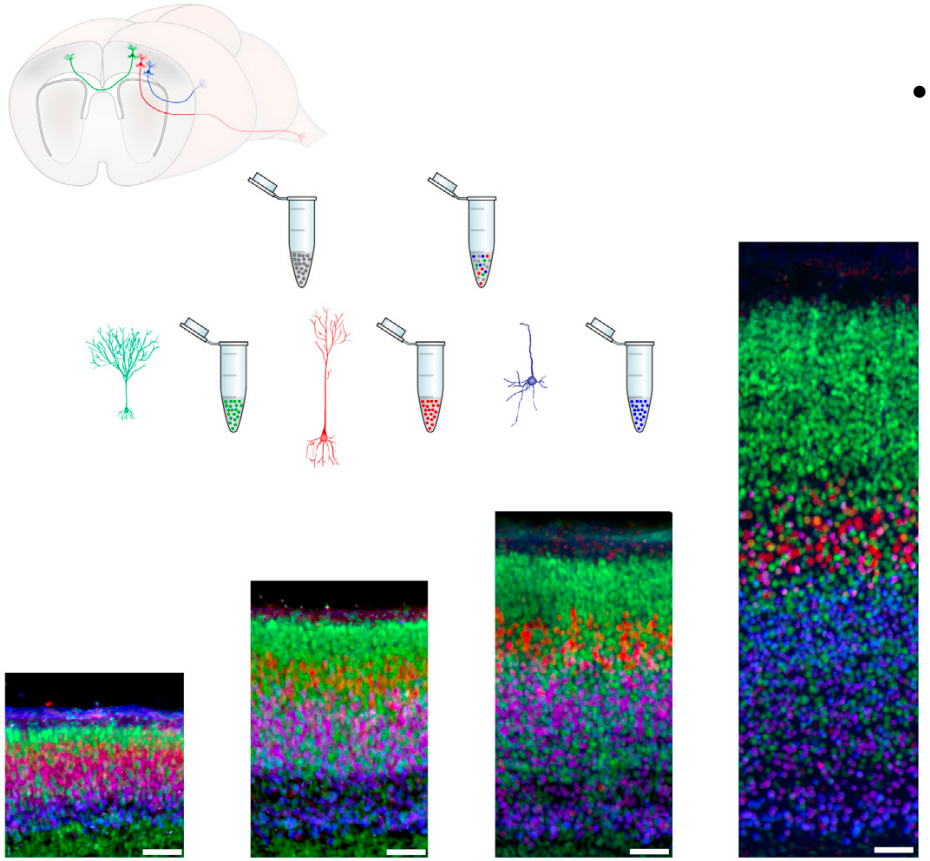
- **Callosal (CPN)**
- **Subcerebral (ScPN)**
- **Corticothalamic/Subplate neurons (CthPN)**

We have conducted isoform-deconvolution based differential RNA-Sequencing on sorted populations of each neuronal subclass at specified timepoints during corticogenesis:

- **E15.5**
- **E16.5**
- **E18.5**
- **P1**

Transcriptome of three different subpopulations of cortical pyramidal neurons:

1-immunostaining against unique combinations of transcription factors (CTIP2, TLE4, and SATB2)
2-fluorescence-activated cell sorting (FACS)
3-whole-transcriptome analyses by massively parallel RNA sequencing



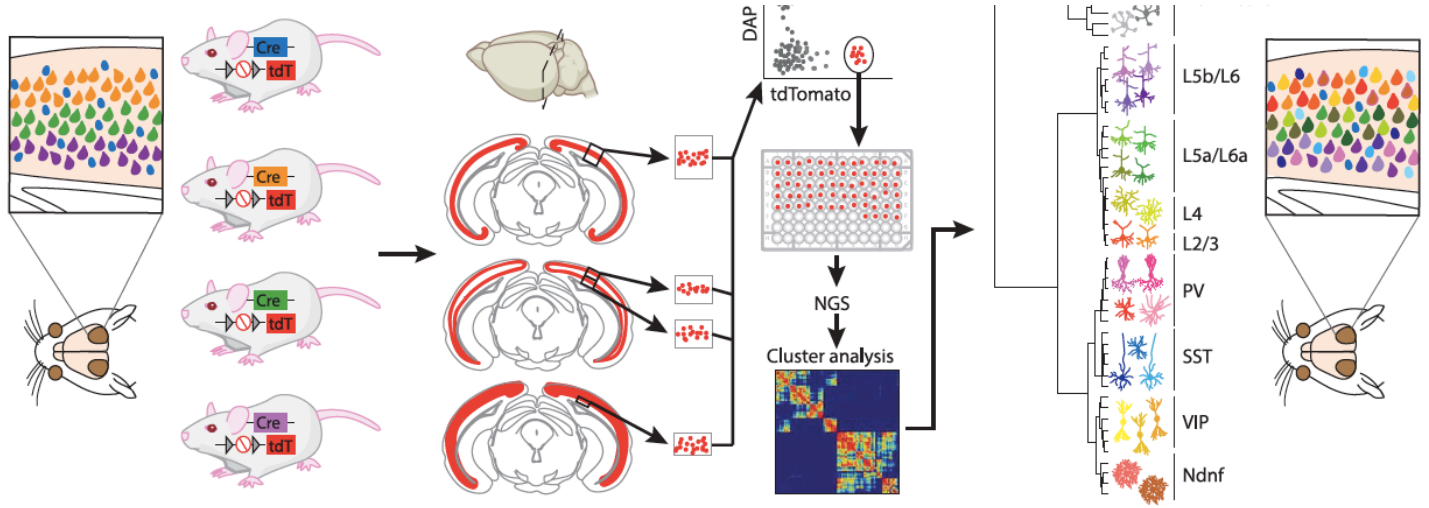
- **subcerebral projection neurons** (ScPN – high CTIP2, low TLE4 and SATB2)
- **callosal projection neurons** (CPNs – high Satb2, no CTIP2 and no TLE4),
- **corticothalamic projection neurons** (CThPNs- high TLE4, moderate CTIP2 and low Satb2)

In total, **8,864** genes identified with significant differential expression

Nature Neuroscience 2016

Adult mouse cortical cell taxonomy revealed by single cell transcriptomics

Bosiljka Tasic[1,2], Vilas Menon[1,2], Thuc Nghi Nguyen[1], Tae Kyung Kim[1], Tim Jarsky[1], Zizhen Yao[1], Boaz Levi[1], Lucas T Gray[1], Staci A Sorensen[1], Tim Dolbeare[1], Darren Bertagnolli[1], Jeff Goldy[1], Nadiya Shapovalova[1], Sheana Parry[1], Changkyu Lee[1], Kimberly Smith[1], Amy Bernard[1], Linda Madisen[1], Susan M Sunkin[1], Michael Hawrylycz[1], Christof Koch[1] & Hongkui Zeng[1]



A large repertoire of Cre driver lines crossed to *loxP* tdTomato (tdT) reporter lines was used to label distinct neuronal populations in the mouse visual cortex.

Specific layers of the primary visual cortex were microdissected from freshly sectioned adult mouse brains, and **single neurons** from these samples were purified by fluorescence-activated cell sorting for use in **single-cell RNA-seq.**

Cluster analysis was conducted agnostic to the reporter line of origin. The resulting clusters were assigned to **49 transcriptionally defined cell types**, 42 of them neuronal, highlighting the **molecular diversity in classes of cortical excitatory and inhibitory neurons**. PV, parvalbumin; SST, somatostatin; VIP, vasoactive intestinal polypeptide; Ndnf, neuron derived neurotrophic factor; NGS, next generation sequencing.

Specification and development of distinct projection neuron subtypes

→ only combinatorial gene expression, and not single molecules, can distinguish one population of PNs from another

→ The combinatorial code is temporally dynamic

→ Transcript expression does not always reflect protein distribution *(e.g Ctip2 mRNA in progenitors + postmitotic SCPN; protein only postmitotic SCPN)*

→ There is regional molecular heterogeneity within defined populations, such that neurons in distinct cortical areas have unique gene signatures

→ Molecular analysis suggests the existence of many more classes of PNs than currently recognized