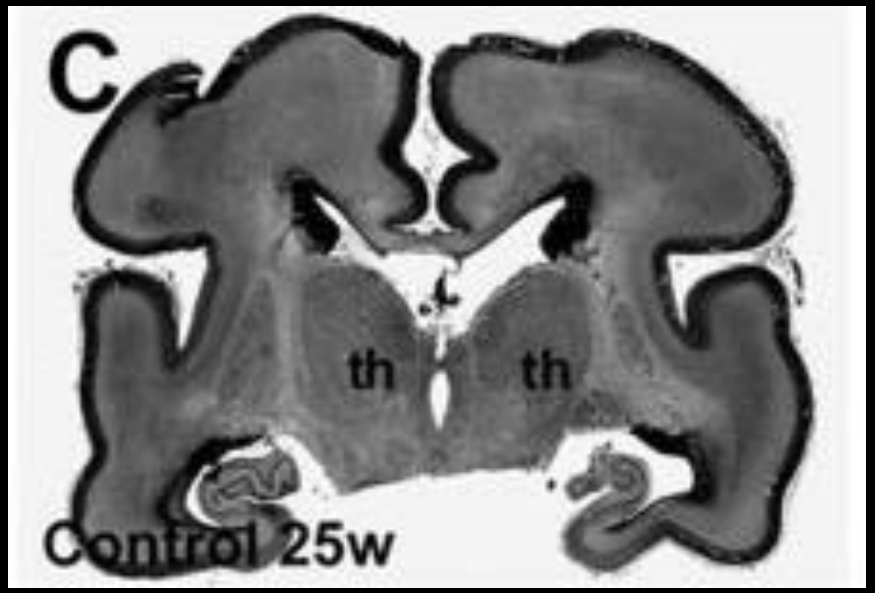
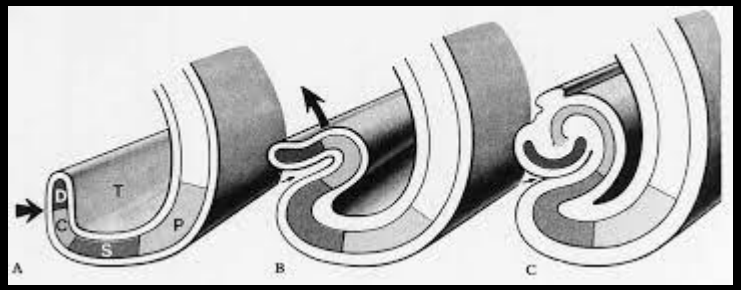
# The development of the hippocampus

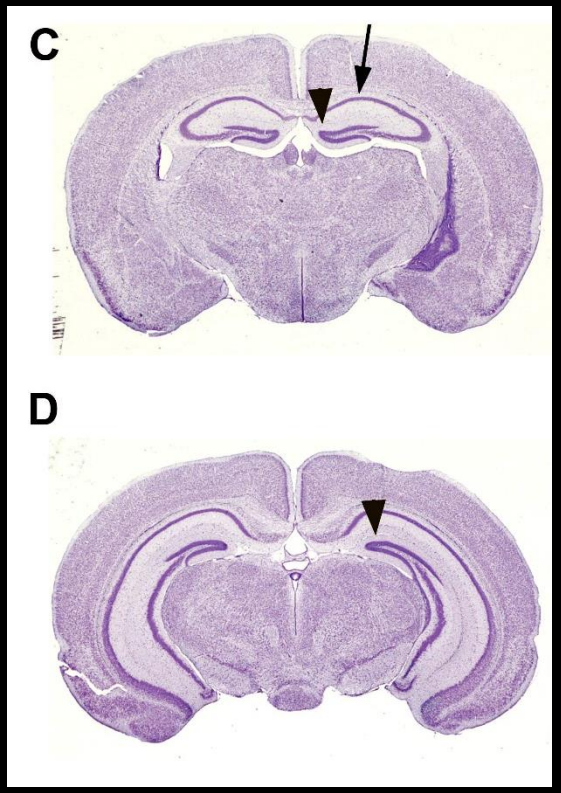


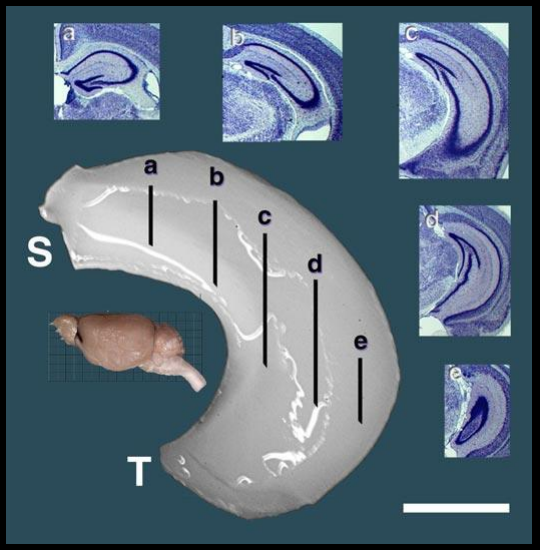




*Leon Kier et al., 1997*

Fiona Francis ifm institut du Fer à moulin Paris

# The mouse hippocampus

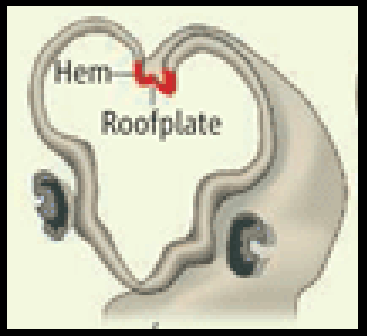



Septo-temporal

# Content today - 1

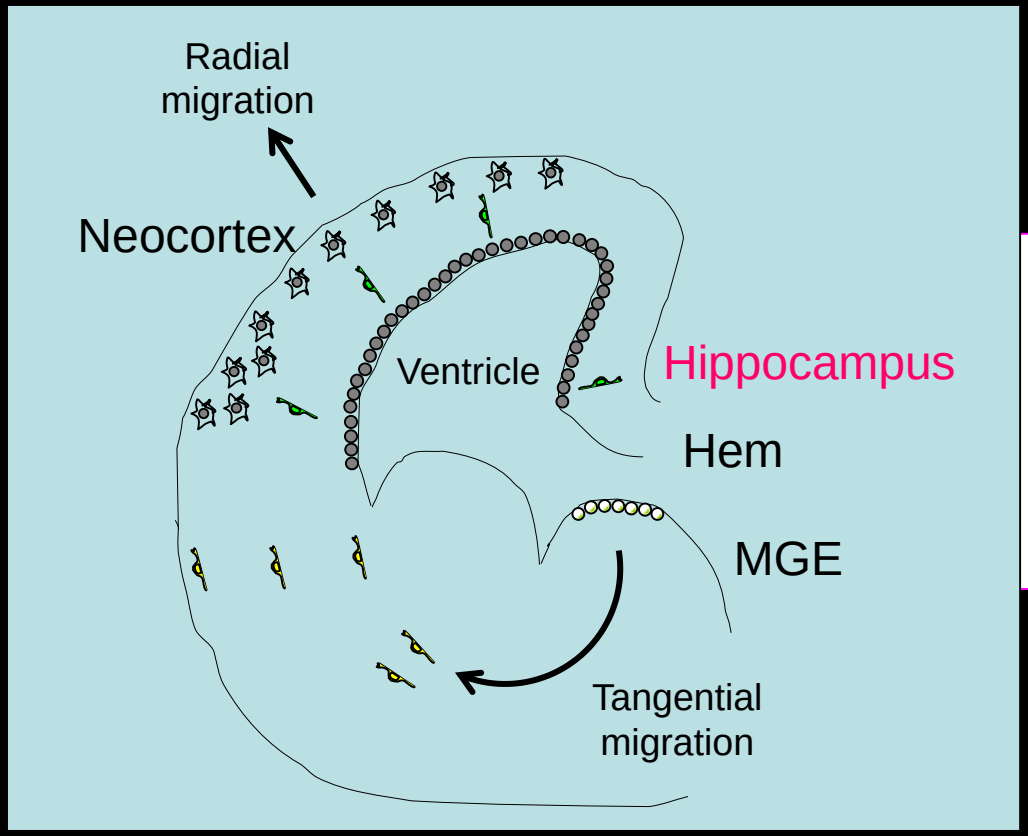
E8.5




The origins of the hippocampus (medial telencephalon, hem, E8.5-10.5) progenitors and pioneer neurons

*Where does it come from?*

# Tissue expansion (mid-embryogenesis)




VZ, ventricular zone
IZ, intermediate zone
CP, cortical plate

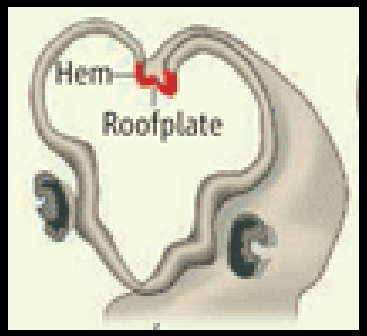
1. Proliferation
2. The generation of neurons
3. Migration
4. Growth of axons and dendrites
5. The formation of synaptic connections

*How does it grow?*

HC and neocortex part of a continuous sheet

# Content today

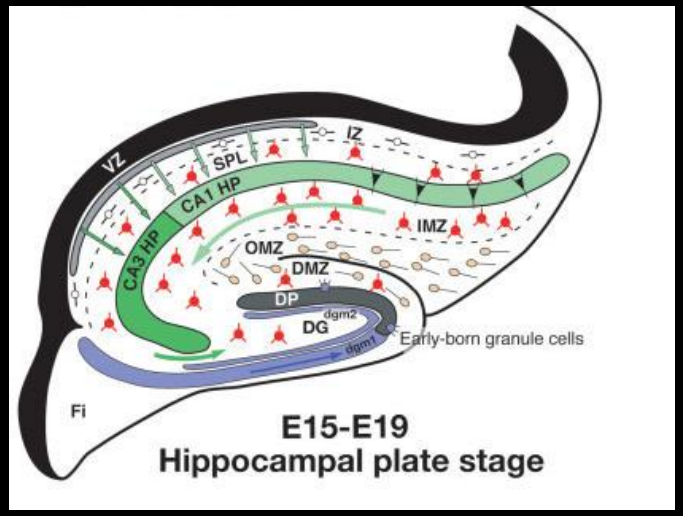
E8.5




The origins of the hippocampus (medial telencephalon, hem, E8.5-10.5) progenitors and pioneer neurons

Neurogenesis, migration and final destination

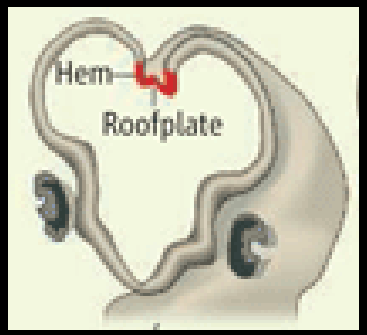
- **CA field**, E11-E16
- Dentate gyrus, E11-postnatal
- Interneurons, E11-E14

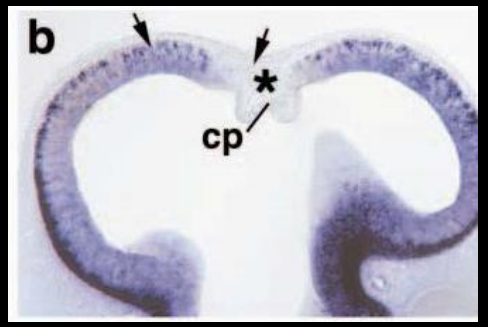



*How do we arrive at this architecture?*

*Danglot et al., 2006*

# The hippocampus develops in the medial telencephalon from E8.5



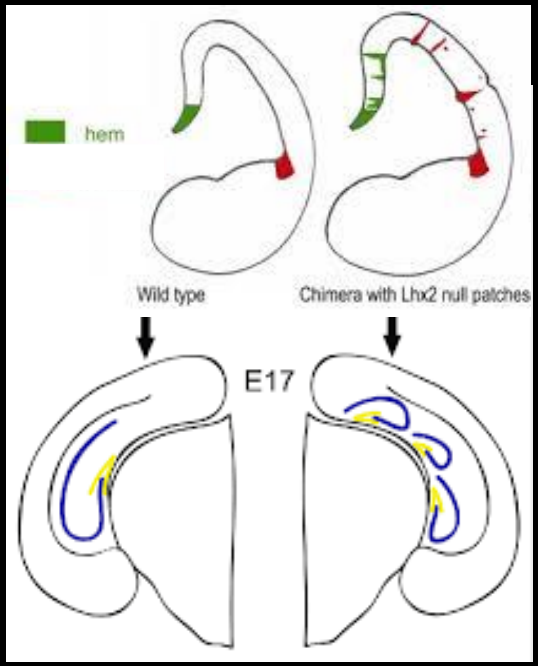




cp = choroid plexus

* = cortical hem

Hem = signaling center

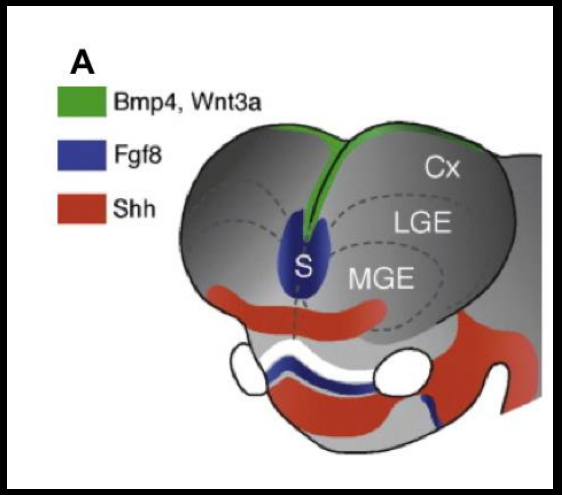
Critical for hippocampal development



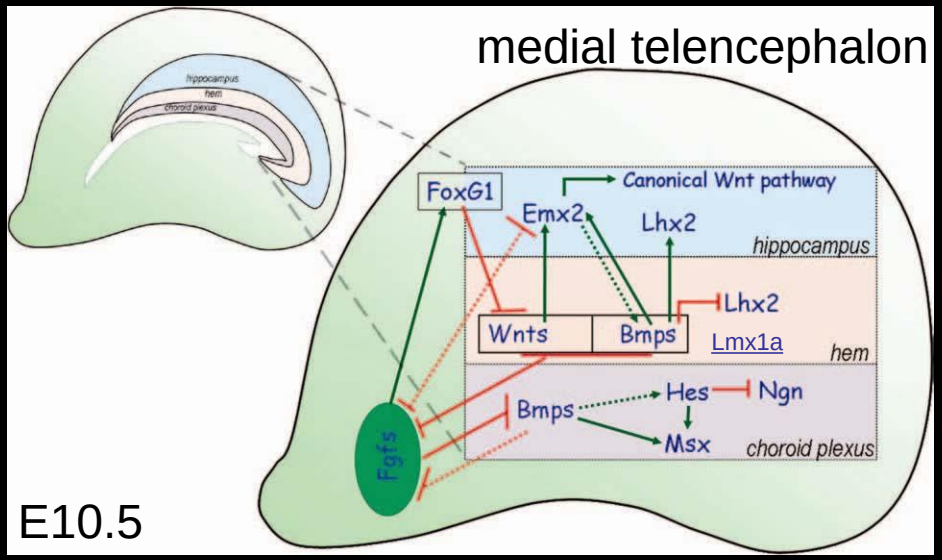

Multiple hem, multiple hippocampi

*Subramanian et al., 2009*

# Hem and position of the hippocampus




Secreted factors

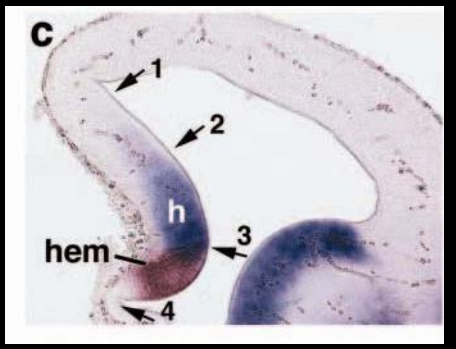



*Multiple genetic interactions*

Hem secretes signaling molecules into surrounding tissue

Mutual interactions between these factors

Wnt3a key molecule expressed in hem from E8.5

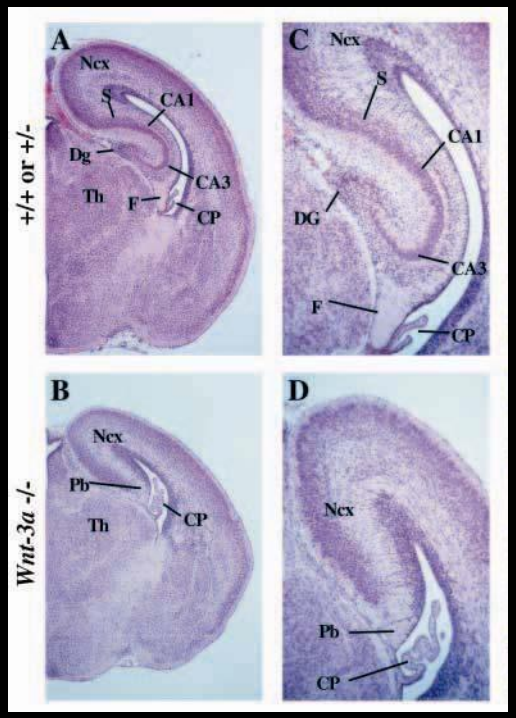



Subramanian and Tole, 2009; Iskusnykh et al., 2023

# Wnt signals – required for HC cell amplification

Wnt signals (‘mitogenic’) from hem are required for regulating the proliferation of adjacent HC progenitor cells

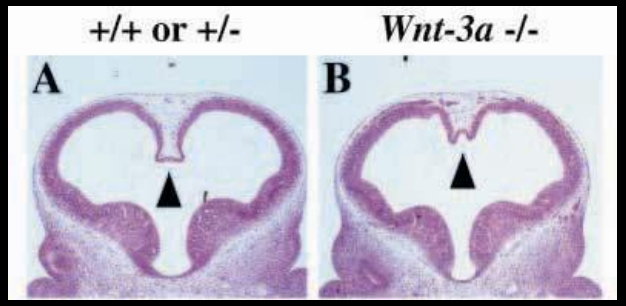
KO – ‘ahippocampal’




E18.5

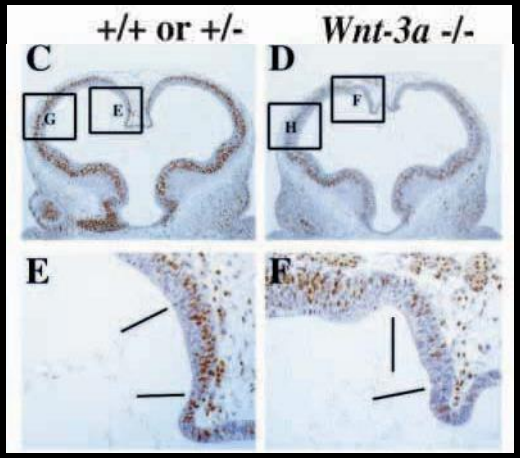
Small residual clusters of CA1, CA3 and DG cells specified (but not many)

Reduction in medial wall




E12.5

Reduction in number of HC progenitors

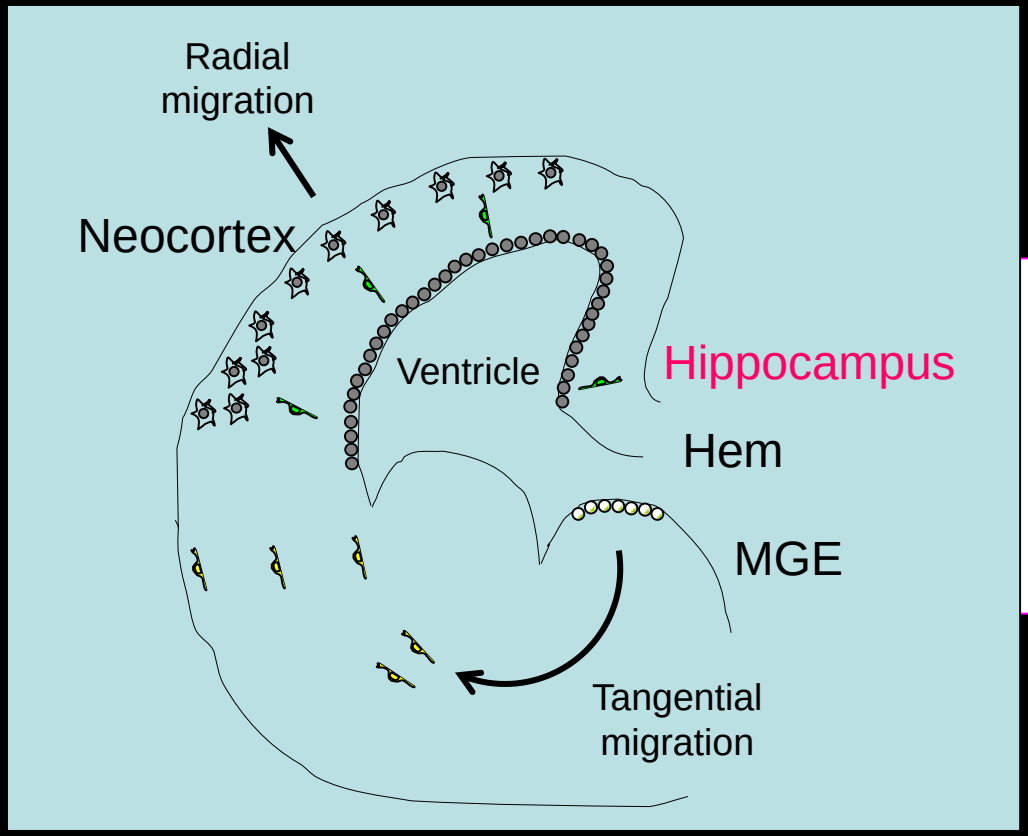



Injection BrdU E10.5
Sacrifice at 30 mins

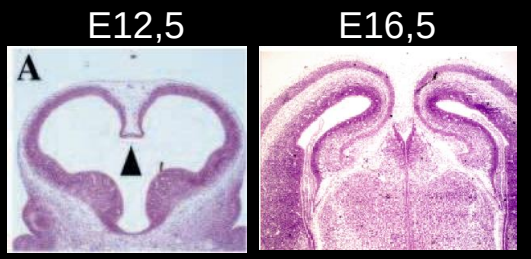
Less BrdU positive cells
(by 26 % P<0.0001)

(Lee et al., 2000)

# Proliferation, neurogenesis, migration




VZ, ventricular zone
IZ, intermediate zone
CP, cortical plate

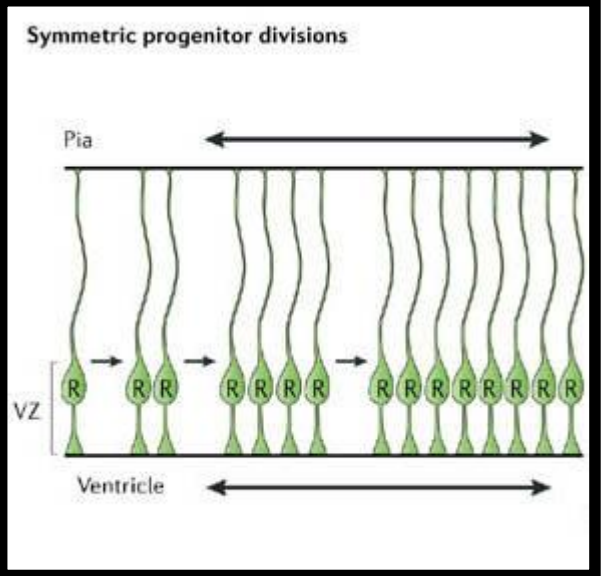



1. Proliferation
2. The generation of neurons
3. Migration
4. Growth of axons and dendrites
5. The formation of synaptic connections

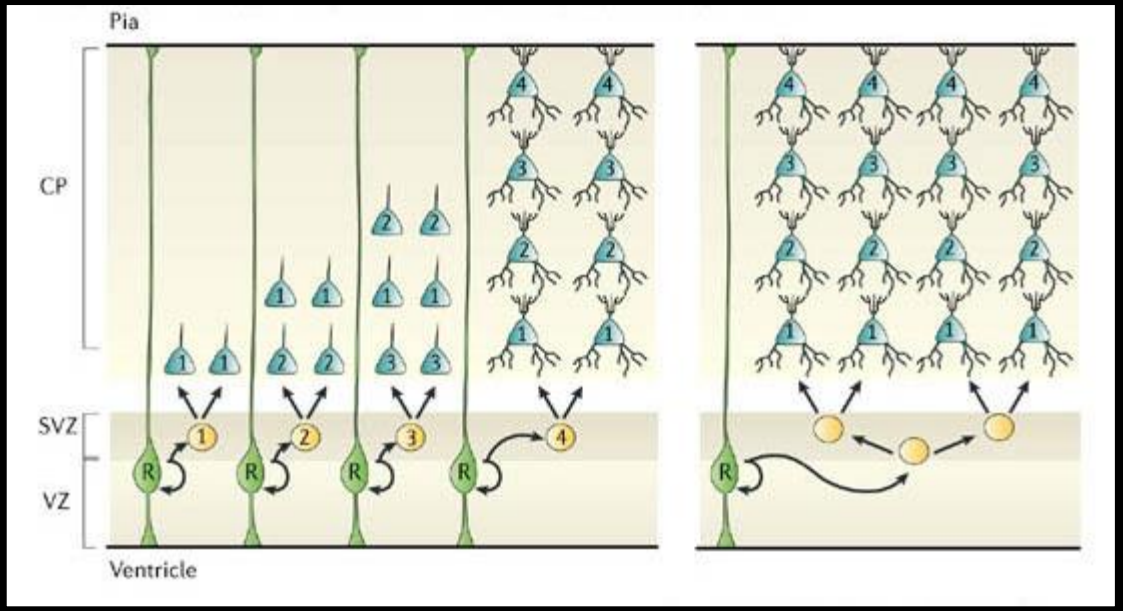
*What are the progenitor cells?*

HC and neocortex part of a continuous apical sheet

# Progenitor types in the developing cortex



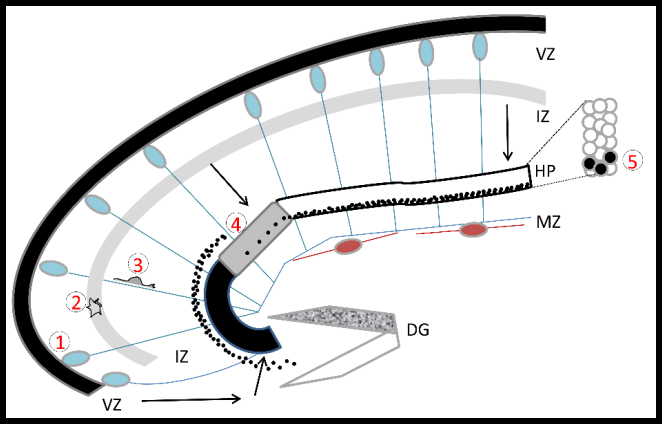

Apical progenitors: radial glial cells




Post-mitotic neurons

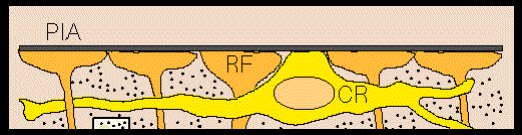
Intermediate progenitors

Kriegstein et al., *Nature Rev Neuroscience* 2006

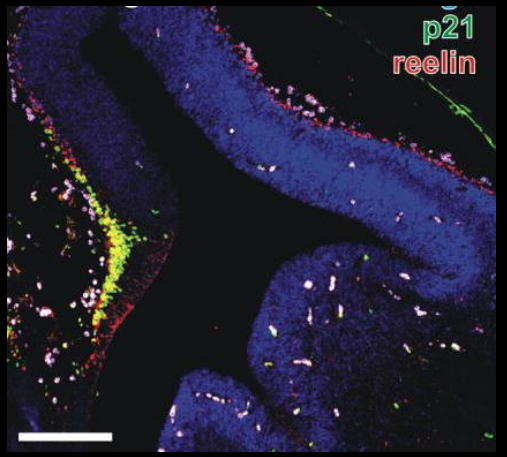



Belvindrah et al, 2014

# Cajal-Retzius neurons


Early superficial pioneer neurons in place before other neurons – ‘mobile signaling centers’

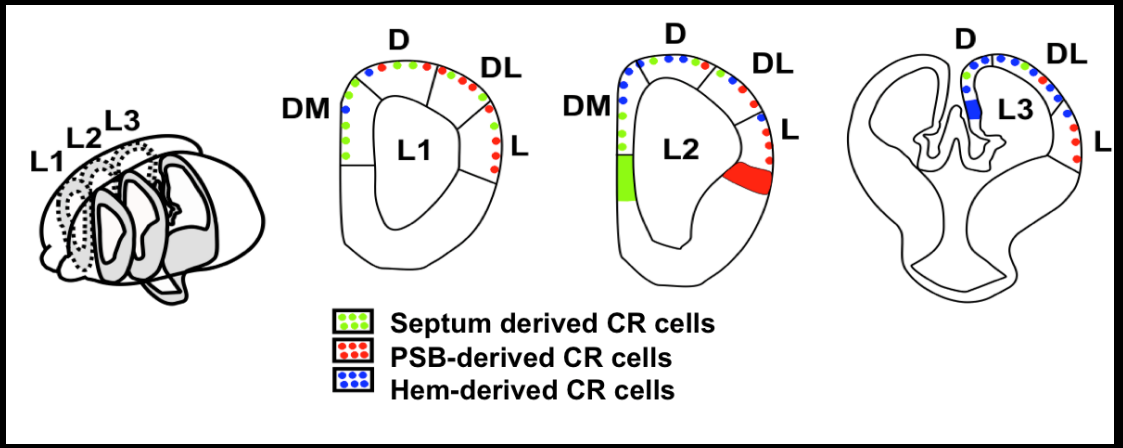



E12.5
p21 newly generated CR cells

Siegenthaler and Miller, 2008

Sources of Cajal Retzius cells

Bielle et al., 2005

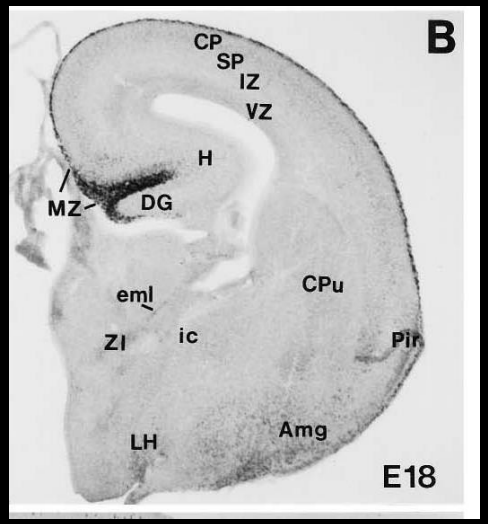


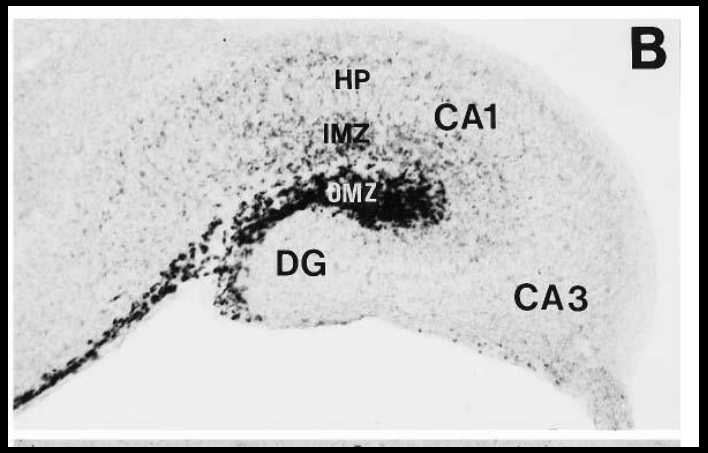

These cells help organize the developing cortical wall

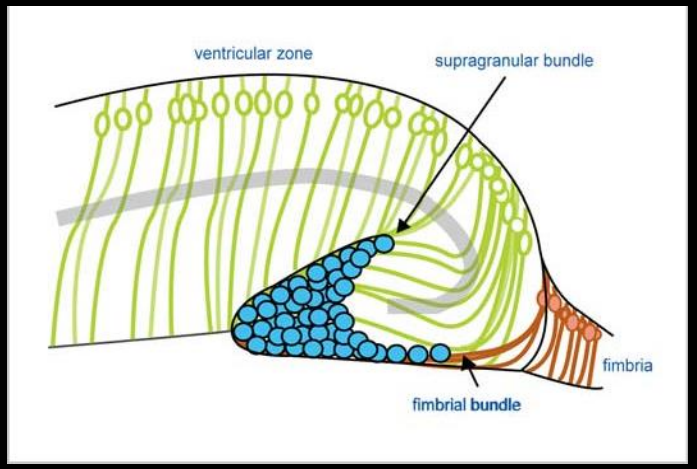
# Reelin, Cajal-Retzius cells and radial glia

Reelin mRNA expression

Alcantara et al., 1998

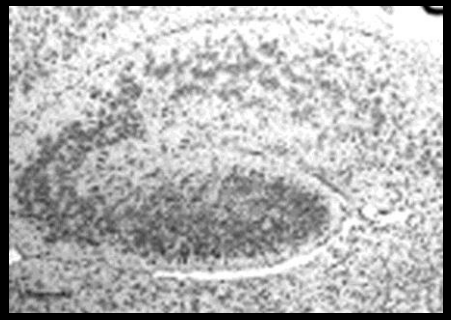


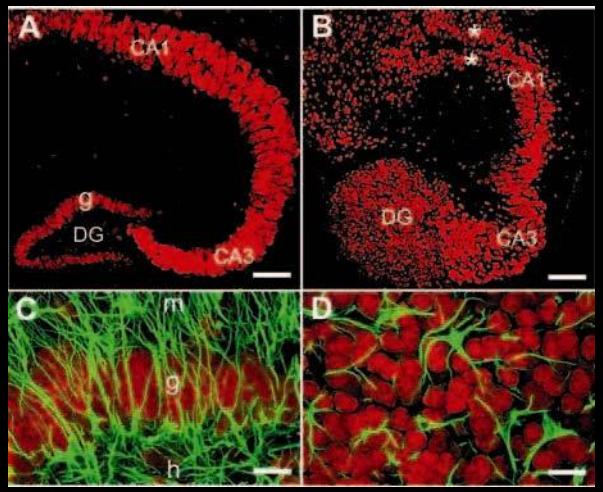
# Reelin and RG cells

Mice with defective Reelin signaling have perturbed radial glial cells



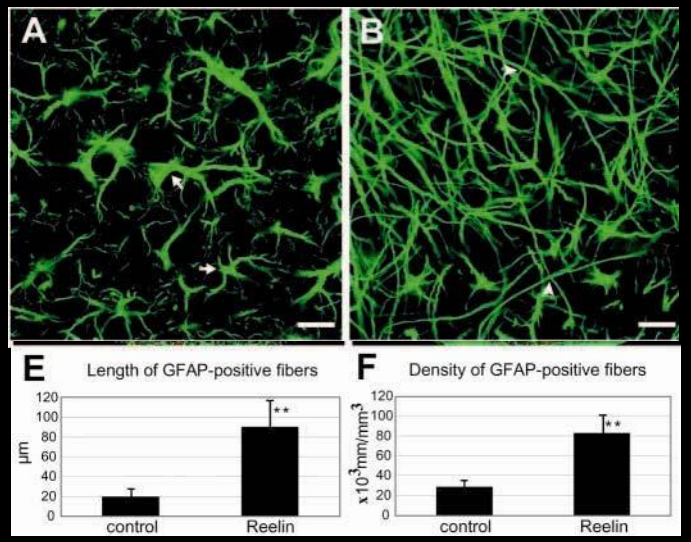
*Reeler*

WT




*RI*

*RI* –




*RI* +

Effect of recombinant Reelin added to culture

Zhao et al., 2004

# Summary 1: Early steps of development

Hippocampus (HC) arises in medial cortex

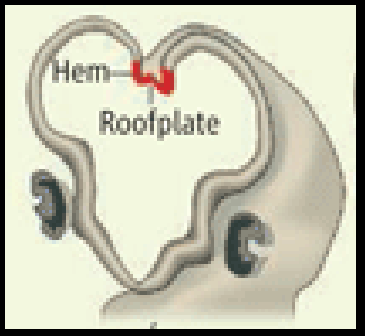
Cortical hem is the hippocampal organizer

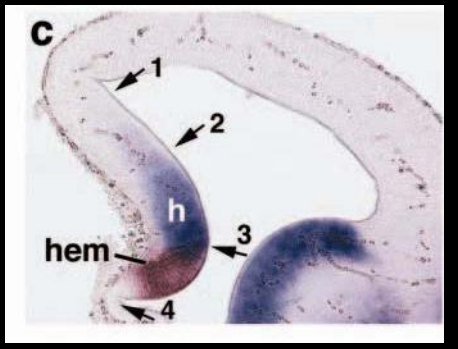
Gene and protein interactions limit boundary of hem/cortex

Wnt signals regulate the expansion of HC progenitors

HC progenitors (radial glia, intermediate progenitors, neuroblasts) give rise to specified cell types (eg CA1 CA2, CA3, DG)

Hem-derived Cajal Retzius neurons are important for radial glial cell integrity and neuron migration



# Neurogenesis and migration

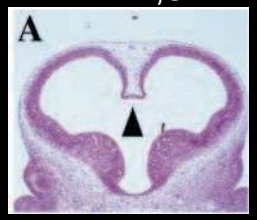
Adult

The origins of the hippocampus (medial telencephalon, hem), progenitors and pioneer neurons

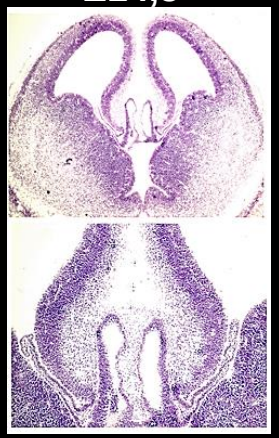
Hippocampal neurogenesis, migration, final destination

- CA field (E11-E16)
- Dentate gyrus granule cells, E11-postnatal
- Interneurons, E11-E14

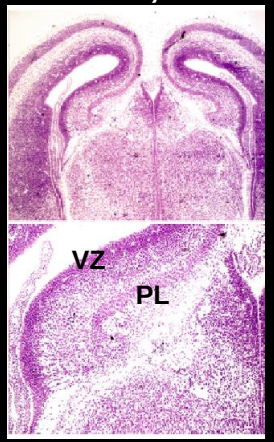
E12,5



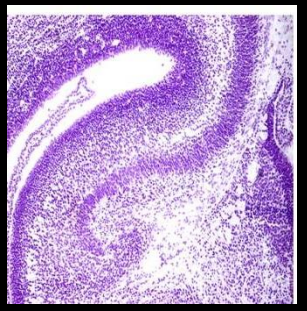

E14,5

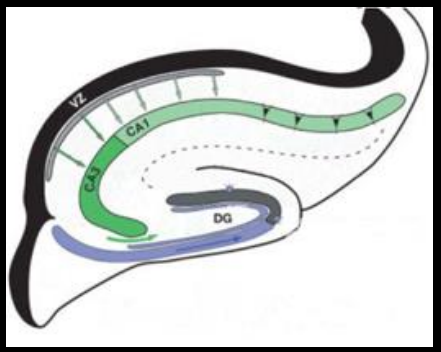


E16,5




E17.5






*Danglot et al., 2006*

# Neurogenesis and birthdates – CA fields

Early birthdating studies* in mice showed that large numbers of pyramidal cells were generated at E14 (similar results obtained in rats)

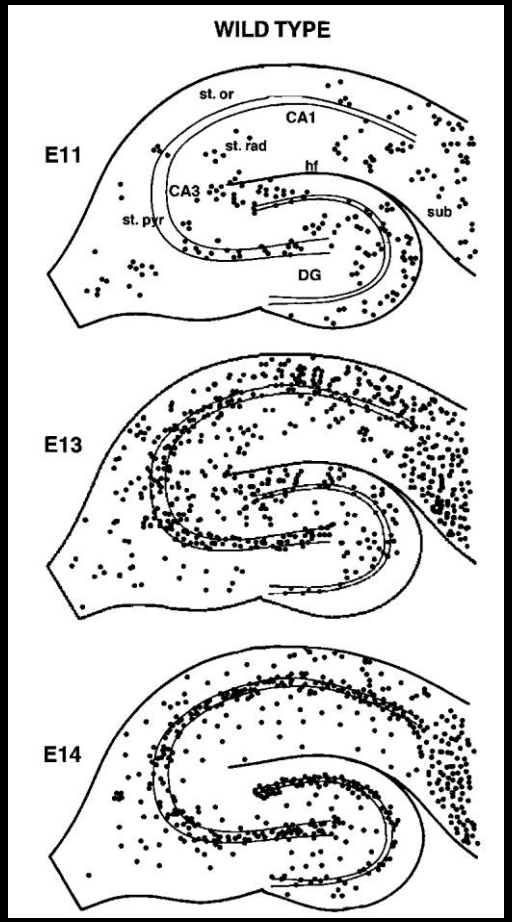
*Nuclei of migrating cells labeled with 3H thymidine

Early studies showed birthdates, peaks of neurogenesis, approximate times required for migration in the different fields

*Angevine, 1965; Stanford and Cowan 1979, Bayer 1980, Altman and Bayer, 1990, Soriano et al., 1986, 1989*

# BrdU birthdating hippocampus

BrdU injections
Sacrifice P0




*BrdU = thymidine analogue*

Fleck et al., 2000

# Neurogenesis and birthdates – CA fields

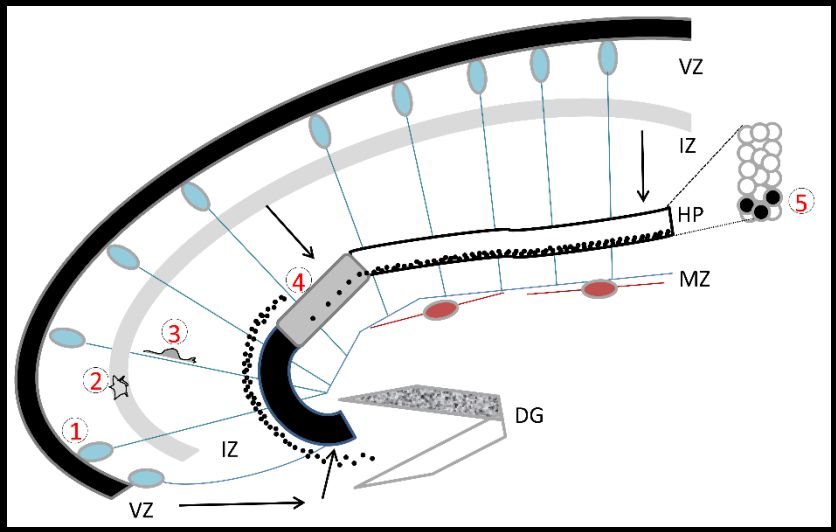
Early birthdating studies* in mice showed that large numbers of pyramidal cells were generated at E14 (similar results obtained in rats)

*Nuclei of migrating cells labeled with 3H thymidine

Many CA3 cells born E13-E15

Many CA1 cells born E14-E16

CA3 neurogenesis peak at E14, CA1 at E15




*Belvindrah et al, 2014*

Early studies showed birthdates, peaks of neurogenesis, approximate times required for migration in the different fields

*Angevine, 1965; Stanford and Cowan 1979, Bayer 1980, Altman and Bayer, 1990, Soriano et al., 1986, 1989*

# Neurogenesis and birthdates – CA fields

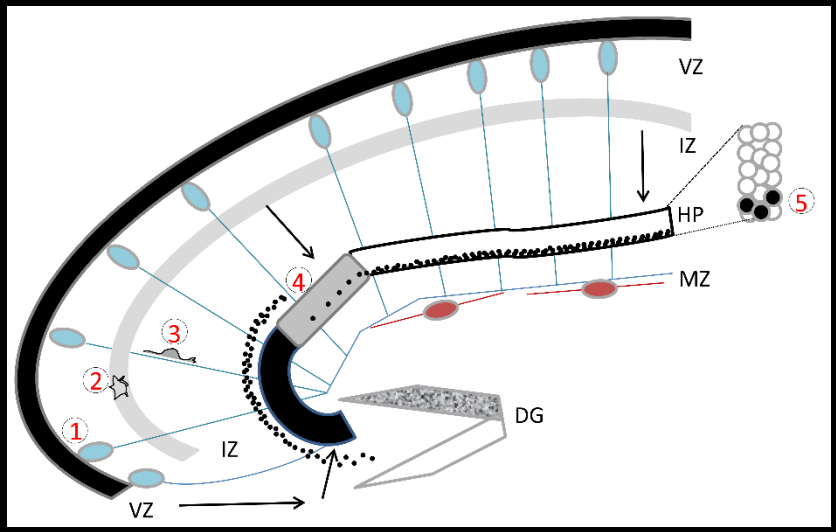
Early birthdating studies* in mice showed that large numbers of pyramidal cells were generated at E14 (similar results obtained in rats)

*Nuclei of migrating cells labeled with 3H thymidine

Many CA3 cells born E13-E15
*4-5 days to reach pyr. layer (cells 'sojourn' in IZ).*

Many CA1 cells born E14-E16
*3-4 days to reach pyr. layer.*

CA3 neurogenesis peak at E14, CA1 at E15

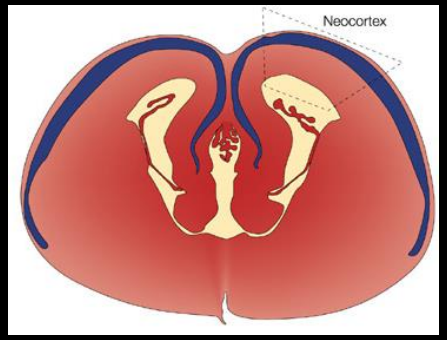



*Belvindrah et al, 2014*

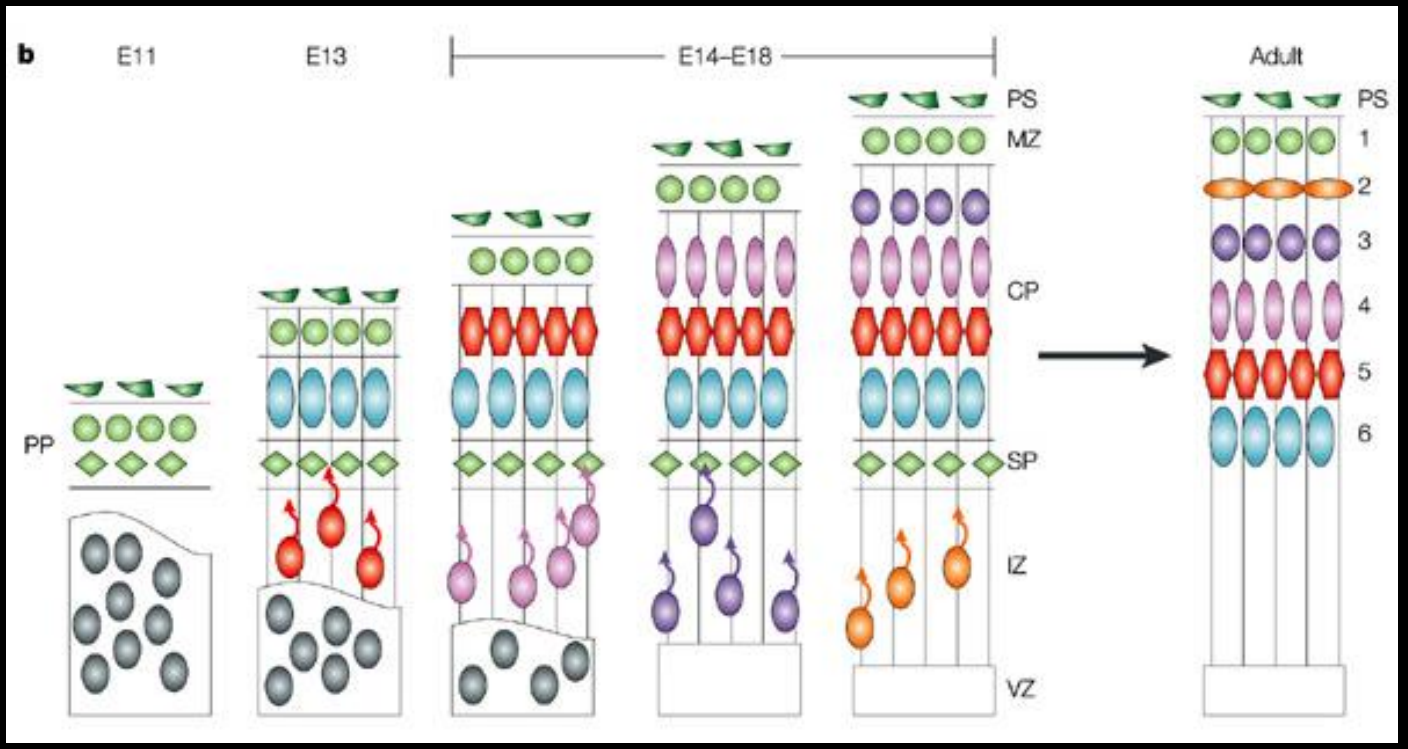
Early studies showed birthdates, peaks of neurogenesis, approximate times required for migration in the different fields

*Angevine, 1965; Stanford and Cowan 1979, Bayer 1980, Altman and Bayer, 1990, Soriano et al., 1986, 1989*

# Neurogenesis and BrdU birthdating




Mouse section




Progenitors in VZ = radial glial cells

*Gupta et al., 2002*

BrdU birthdating reveals neuron layering develops by inside-out lamination

Based on waves of neuronal migration

Thompson et al, 2008; Xu et al, 2014, Khalaf-Nazzal et al, 2017

# Layering and cell heterogeneity in the CA cell layer

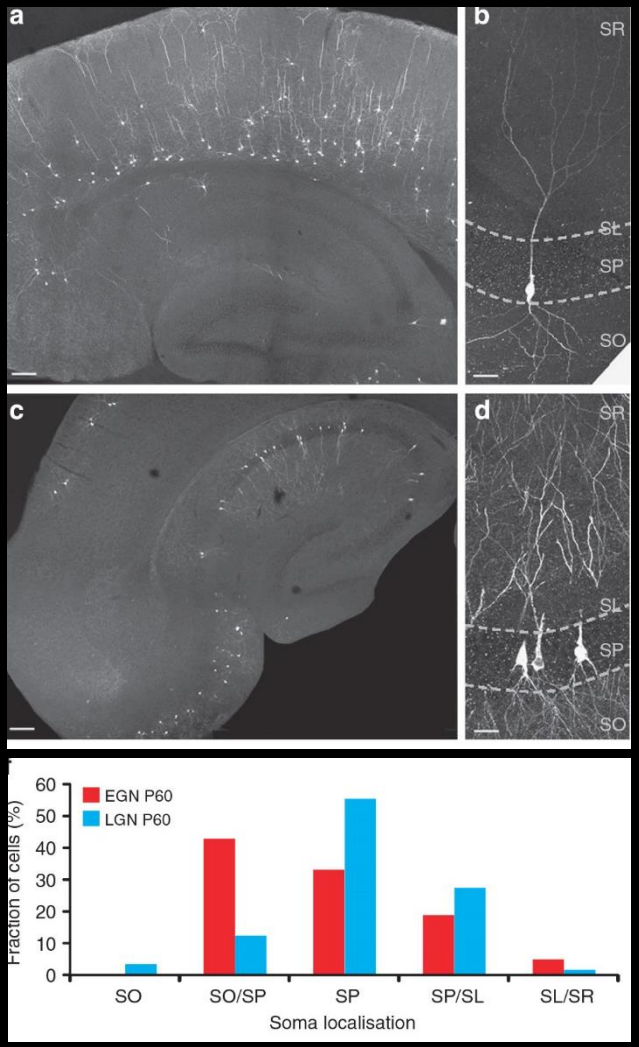
**Strata**

*Radiatum (SR)*

*Laconusum moleculare (SL)*

*Pyramidale (SP)*

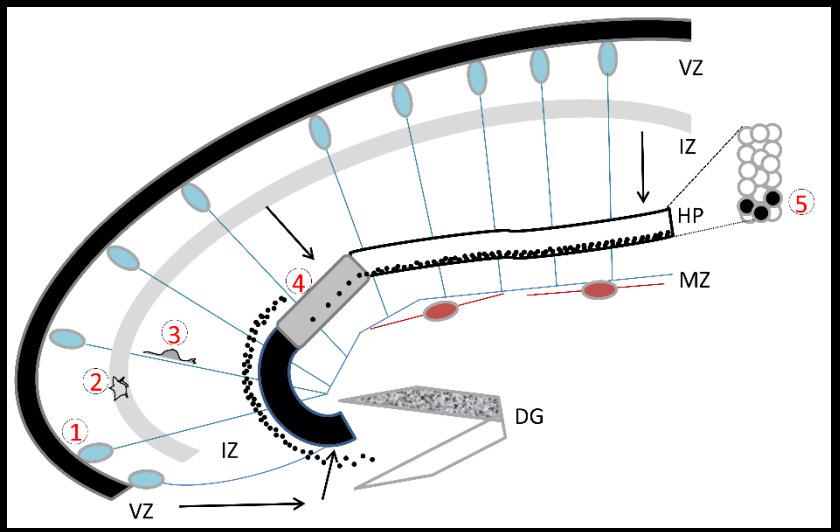
*Oriens (SO)*




Even though pyramidal cell layer is compact…

Many early-generated cells closer to *st. oriens*

Later-generated cells generally more superficial




*Belvindrah et al, 2014*

*EGN, early generated neuron; LGN, late generated neuron*

*Marissal et al, 2012; Hunt et al, 2018; Cembrowski and Spruston, 2019*

# CA1 pyramidal cell diversity is rooted in the time of neurogenesis

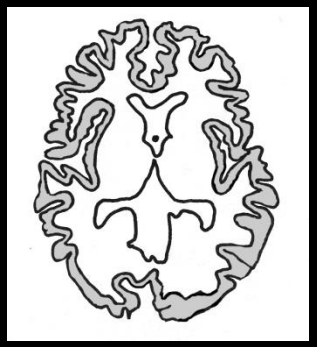
Birthdate is a crucial determinant of cell heterogeneity, not solely explained by radial gradient

*Genetic fate-mapping*
*Synaptic inputs, intrinsic properties*
*Dendritic morphologies, tracing*
*cFos after exploration*

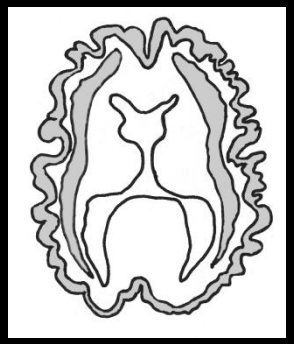
*Cavalieri et al., 2021 ELife*

# Mouse models of ‘lissencephaly’

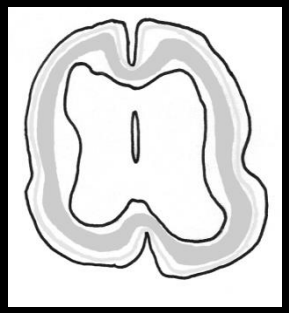
**abnormal hippocampal lamination**



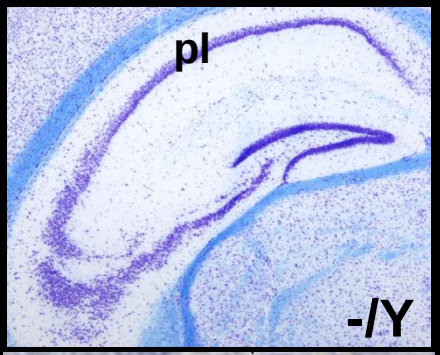
Control



Subcortical band heterotopia

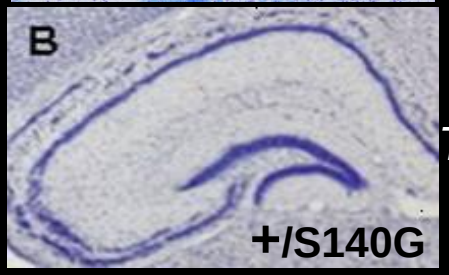


Lissencephaly



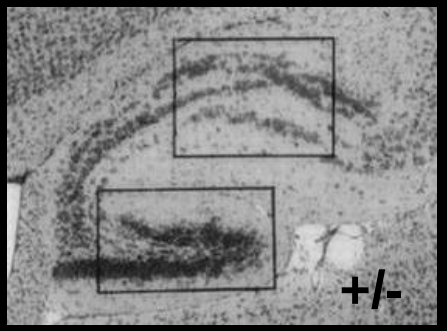

*Dcx*

No major isocortical abnormalities
Spontaneous limbic seizures
(Nosten-Bertrand et al., 2008)




*Tuba1a*

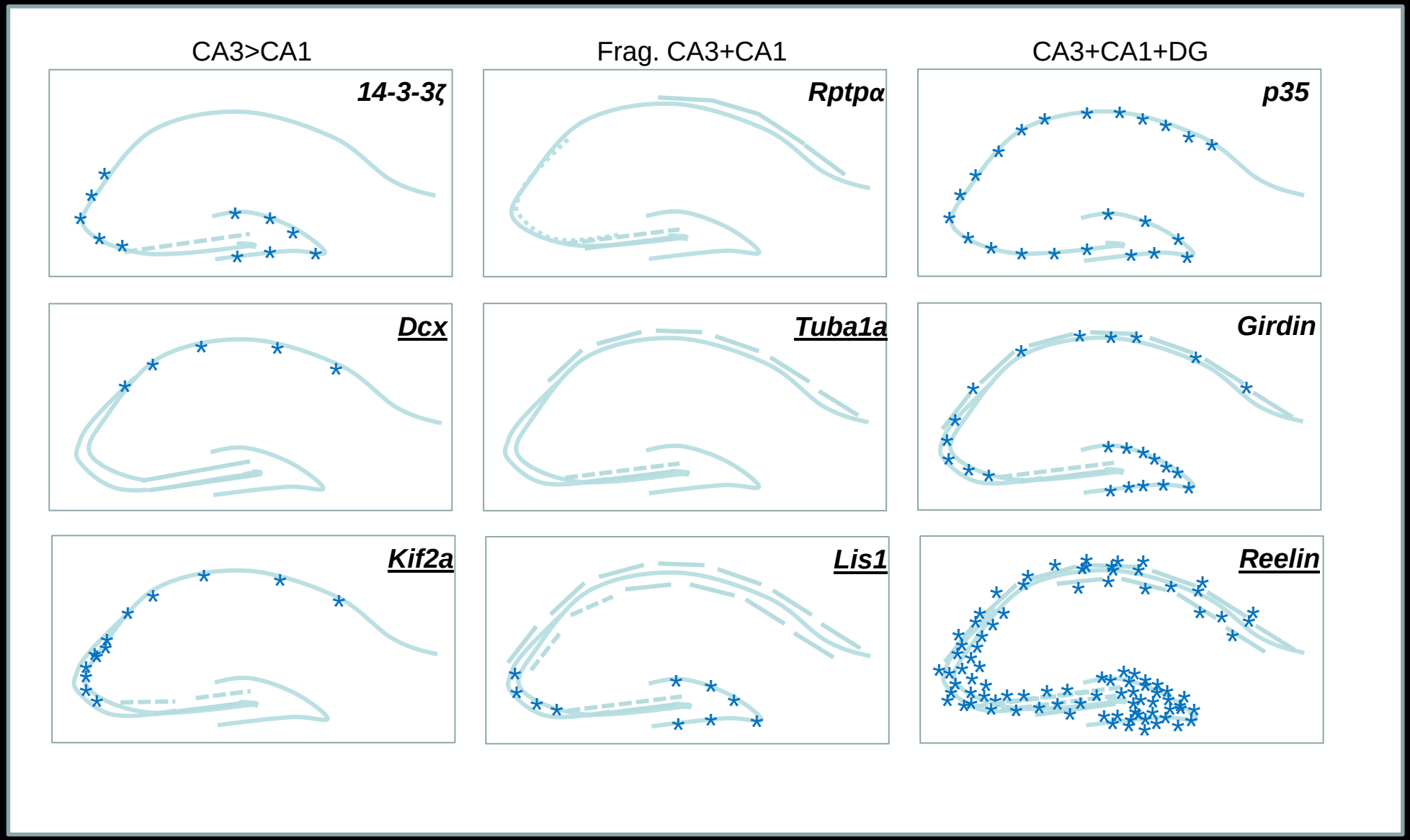
Mild isocortical abnormalities
?




*Lis1*

Mild isocortical abnormalities
Spontaneous limbic seizures
(Hirotsune et al., 1998; Fleck et al., 2000)

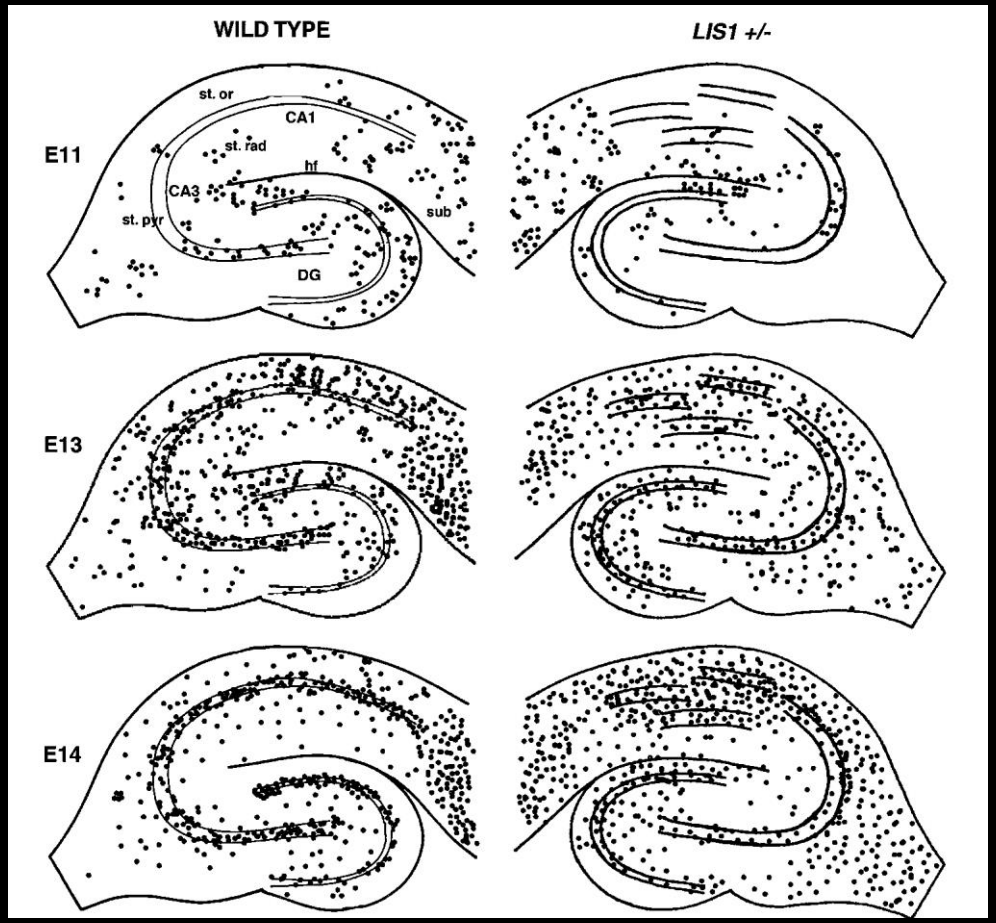
# Hippocampal ‘lamination’ mutants




*Belvindrah et al, 2014*

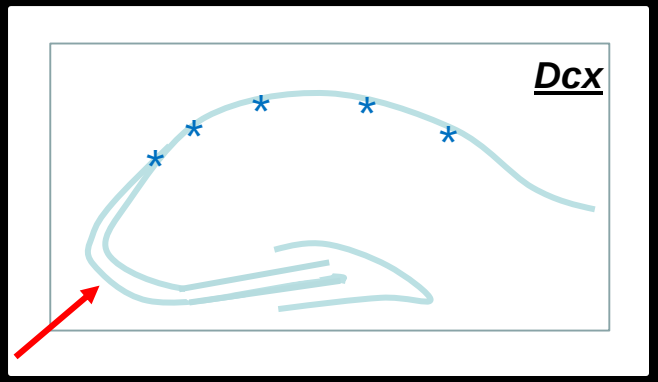
# *Lis*1 mutants – perturbed radial migration

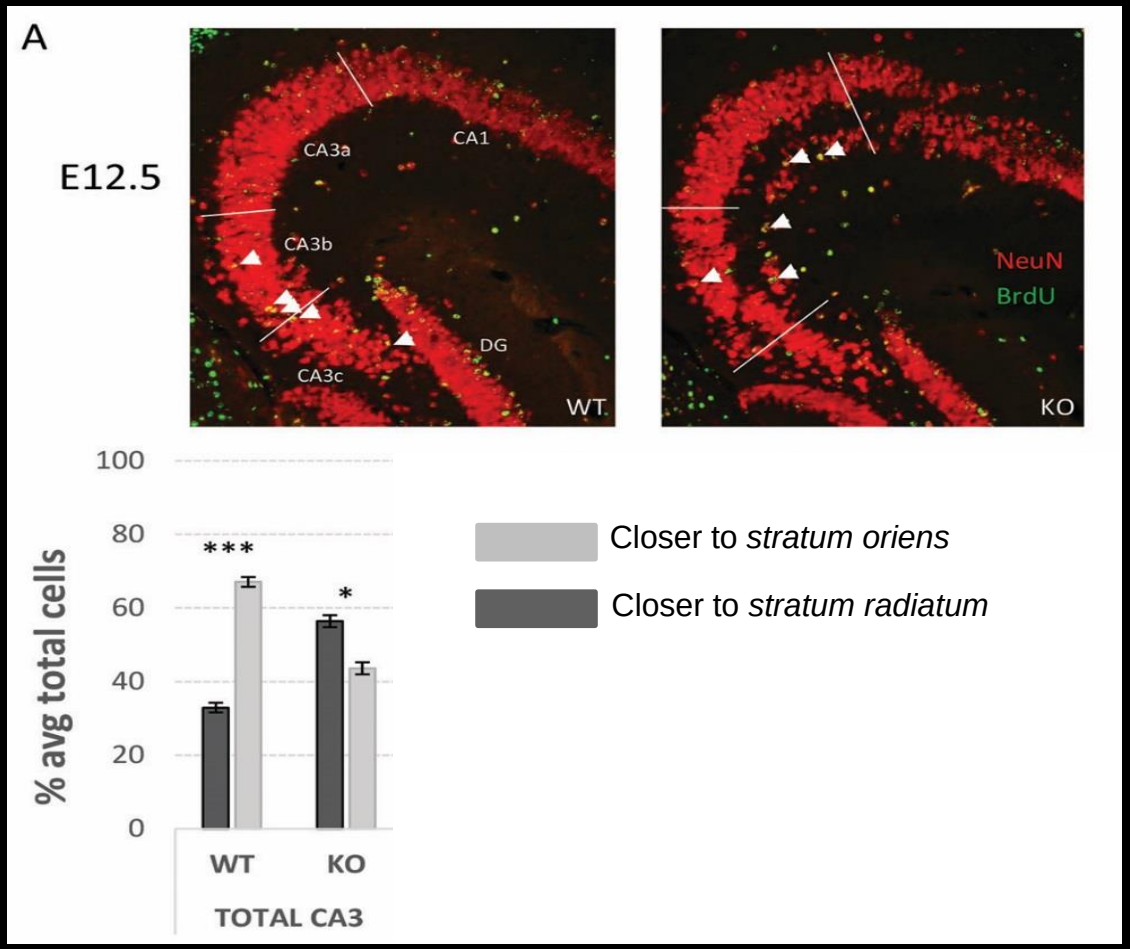
BrdU injections




Fleck et al., 2000

# *Dcx* mutants – perturbed CA3 region



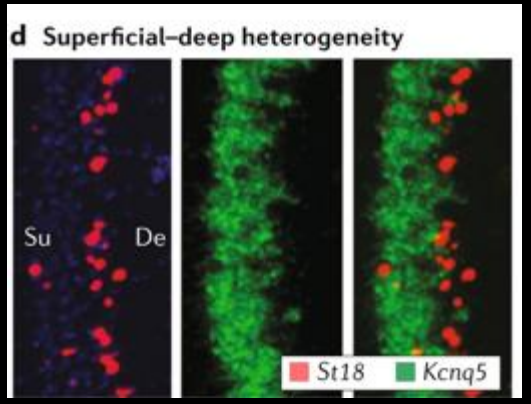




Mutants: Early born neurons are more superficial (instead of deep)

*Khalaf-Nazzal et al, 2017 (Lis1 mutants, d'Amour et al., 2020)*

# Radial axis and cell heterogeneity in the CA cell layer

Genetic markers show heterogeneity

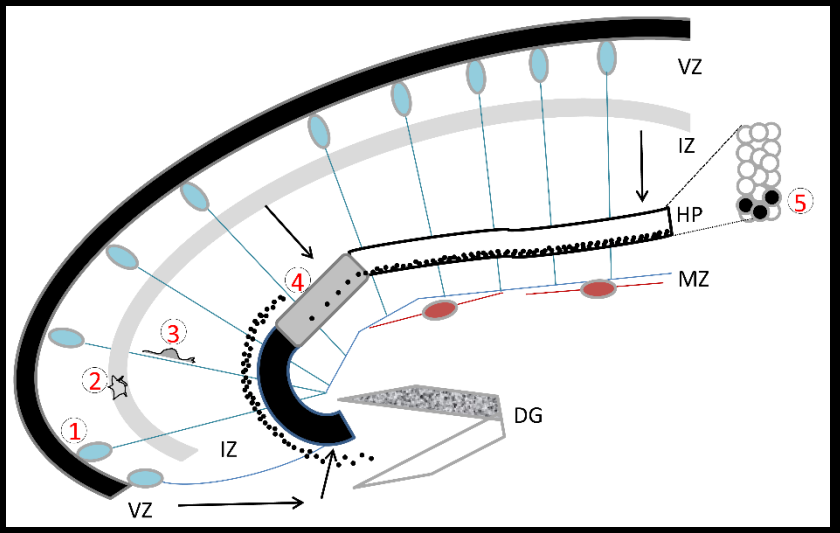



‘Outer-boundary’ neurons

*St18* = early-generated

Early-generated cells closer to *st. oriens*

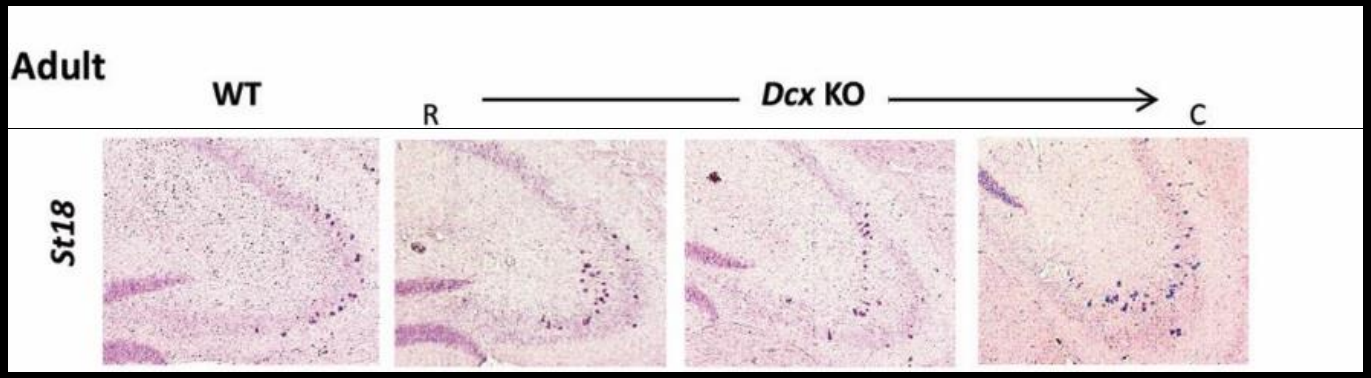
Later-generated cells generally more superficial



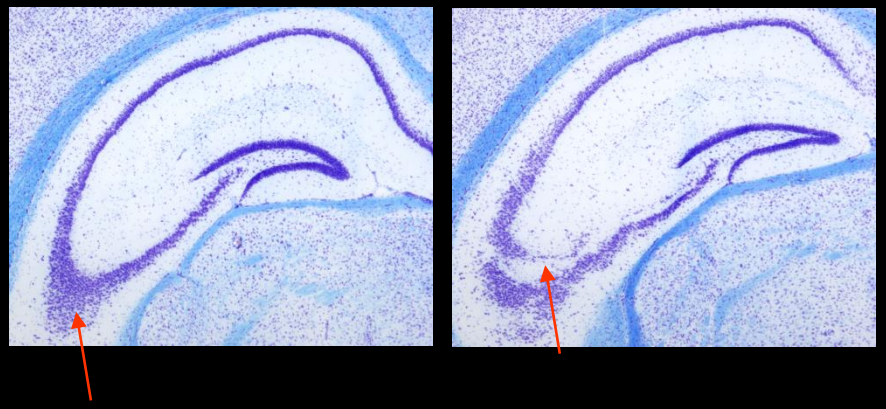

*Belvindrah et al, 2014*

*Marissal et al, 2012; Hunt et al, 2018; Cembrowski and Spruston, 2019*

# *Dcx* mutants – early-born neurons are superficial




*St18* in situ hybridisation



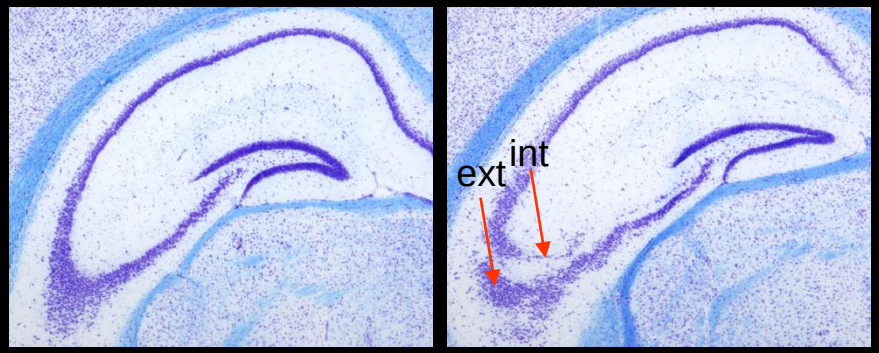
Consequences?

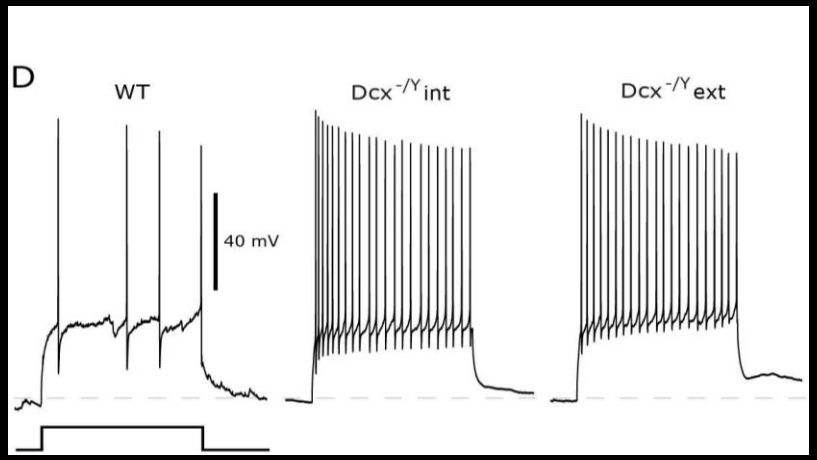
Abnormal lamination = abnormal migration

Dcx plays a role in neuronal migration (linked to microtubule cytoskeleton)

*Khalaf-Nazzal, Stouffer et al, 2017*

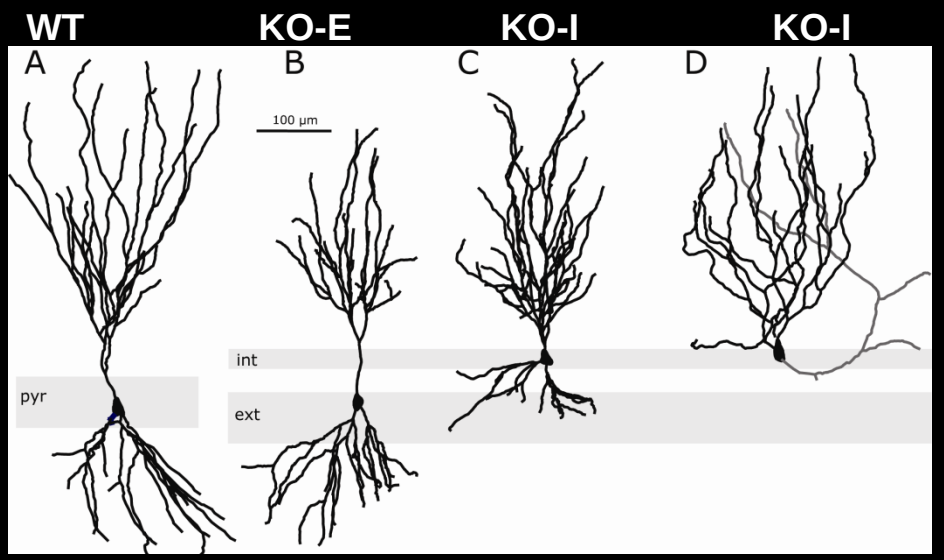
# *Dcx* KO CA3 pyramidal cell layering abnormalities




Bazelot et al., 2012

Excitability

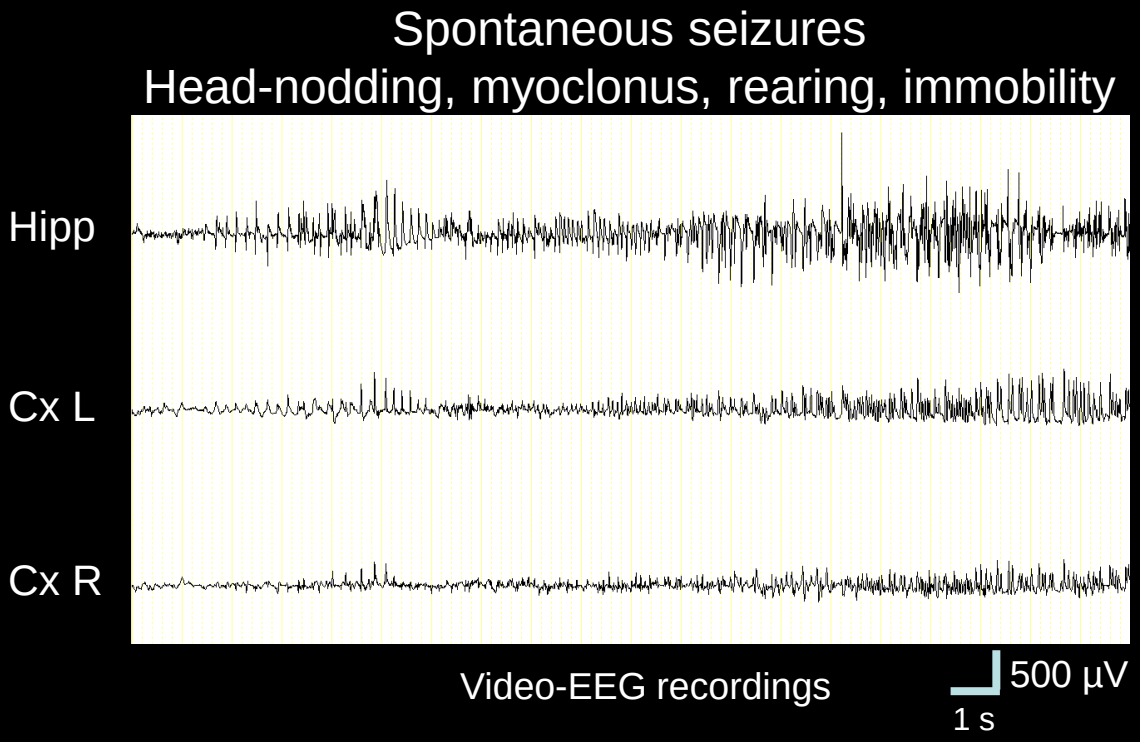


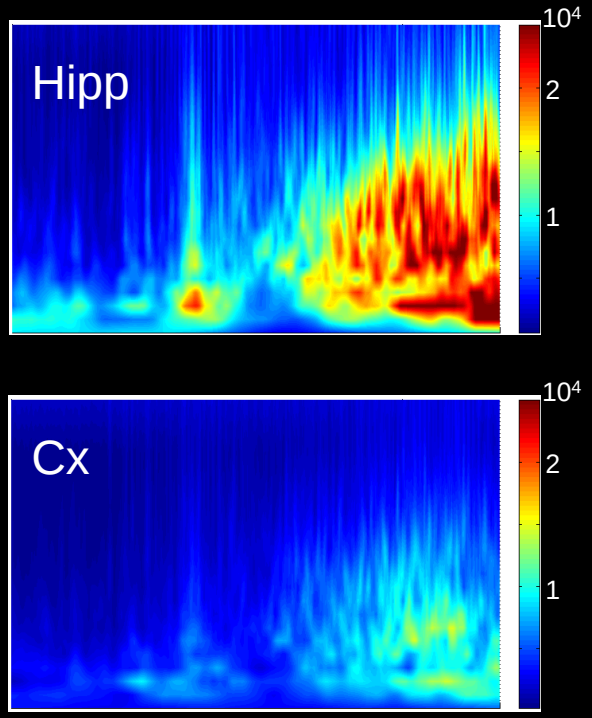

Group of R. Miles, CRICM, La Pitié-Salpêtrière, Paris

Abnormal morphology

Mice have spontaneous limbic seizures

# *Dcx* knockout: spontaneous seizures are initiated in the hippocampus




*Nosten-Bertrand et al (2008)*

# Summary 2: CA cell neurogenesis

Many CA1 neurons born E14-E16, 3-4 days to reach pyr. layer

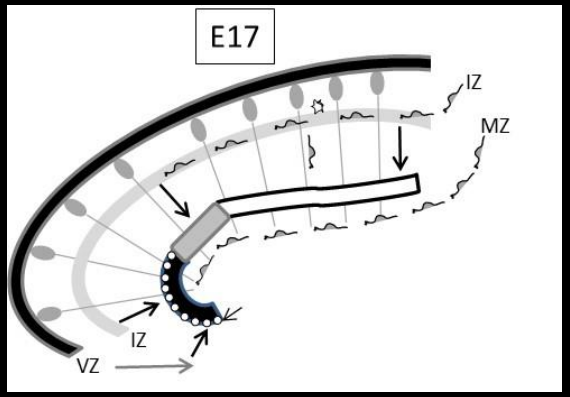
Many CA3 neurons born E13-E15, 4-5 days to reach pyr. layer (cells ‘sojourn’ in IZ)

CA3 pyramidal cells longer curved route to arrive in compact pyramidal layer

Early-generated neurons on the outside (closer to the *stratum oriens)*

Late-generated neurons more superficial

Hippocampal lamination mouse mutants often show neuron disorganization & epilepsy



# Neurogenesis and migration

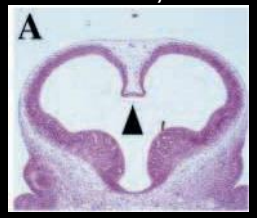
Adult

The origins of the hippocampus (medial telencephalon, hem), progenitors and pioneer neurons

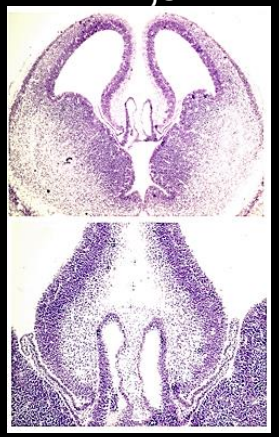
Hippocampal neurogenesis, migration, final destination

- CA field (E11-E16)
- Dentate gyrus granule cells, E11-postnatal
- Interneurons, E11-E14

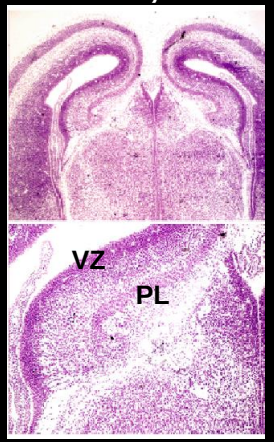
E12,5



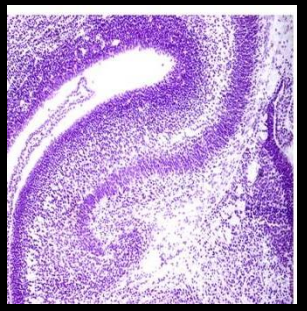

E14,5

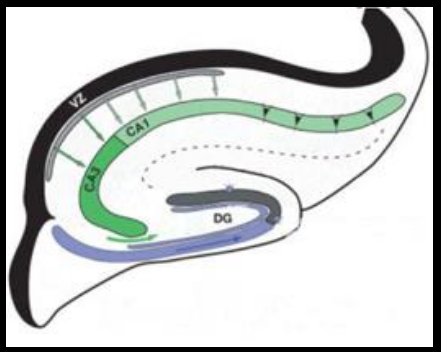


E16,5




E17.5

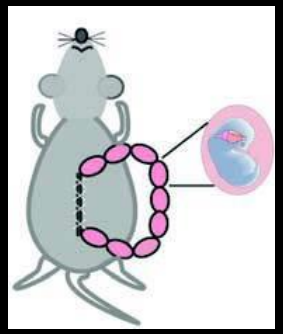


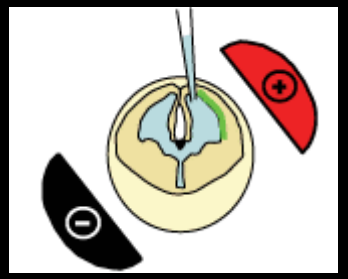



*Danglot et al., 2006*

# Electroporation *in utero*

## An essential tool for studying cortical development



Intracerebro-ventricular injection of plasmid
Electroporation (tweezer electrodes)
Embryos continue to develop *in utero*

Expression of fluorescent markers

Overexpression and rescue of mutated genes

Rapid inactivation of genes (RNAi)

# *In utero* electroporation - hippocampus

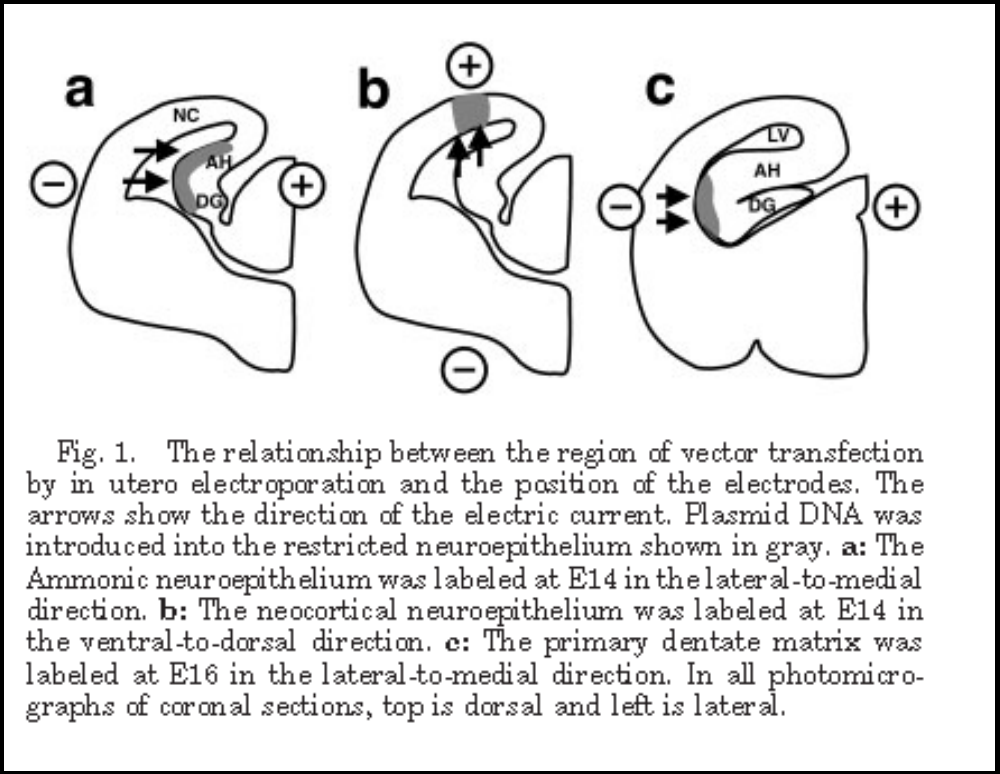
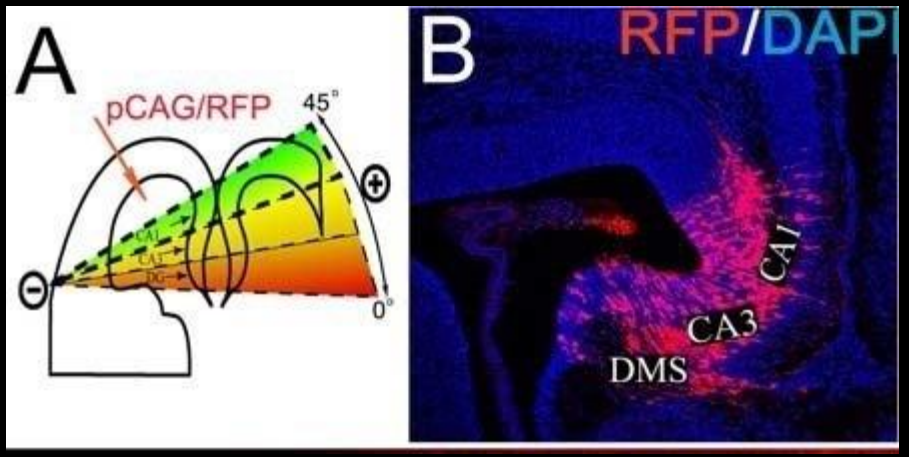
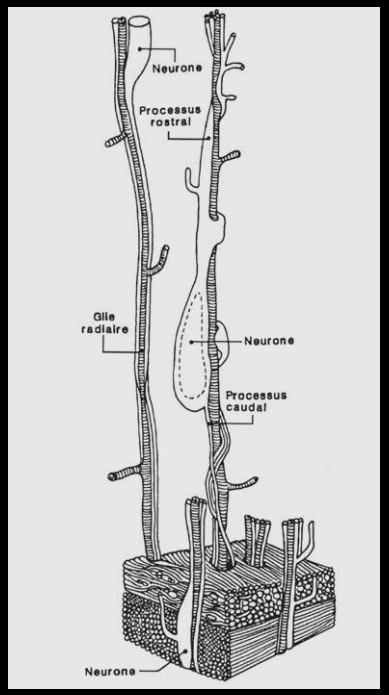


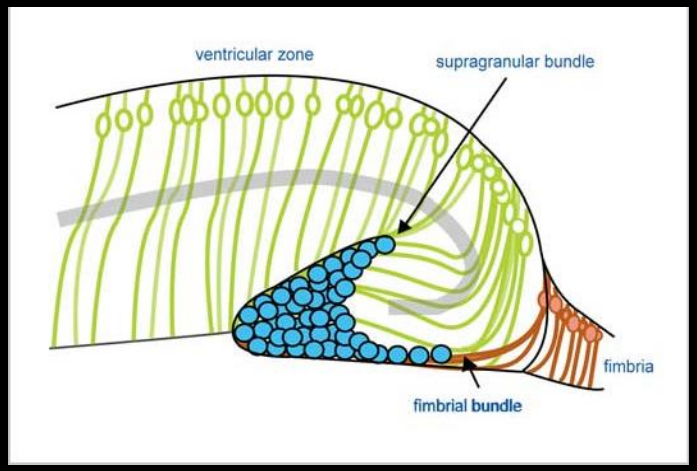

Fig. 1. The relationship between the region of vector transfection by in utero electroporation and the position of the electrodes. The arrows show the direction of the electric current. Plasmid DNA was introduced into the restricted neuroepithelium shown in gray. **a:** The Ammonic neuroepithelium was labeled at E14 in the lateral-to-medial direction. **b:** The neocortical neuroepithelium was labeled at E14 in the ventral-to-dorsal direction. **c:** The primary dentate matrix was labeled at E16 in the lateral-to-medial direction. In all photomicrographs of coronal sections, top is dorsal and left is lateral.

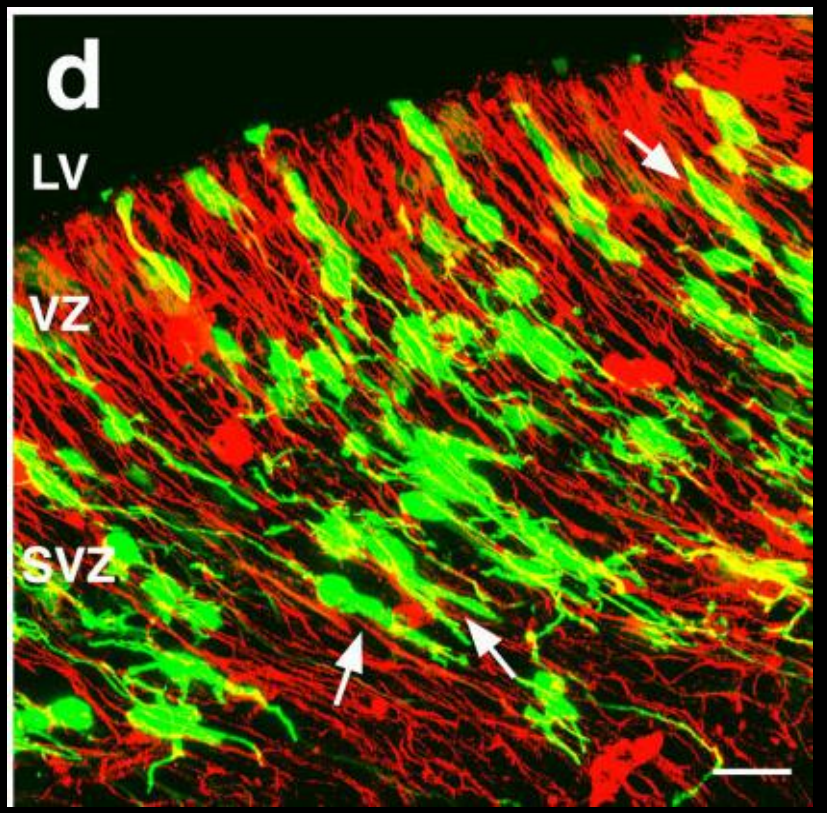



Nakahira and Yuasa, 2005; Navarro-Quiroga et al., 2007

# RG cell processes and migration in the CA1



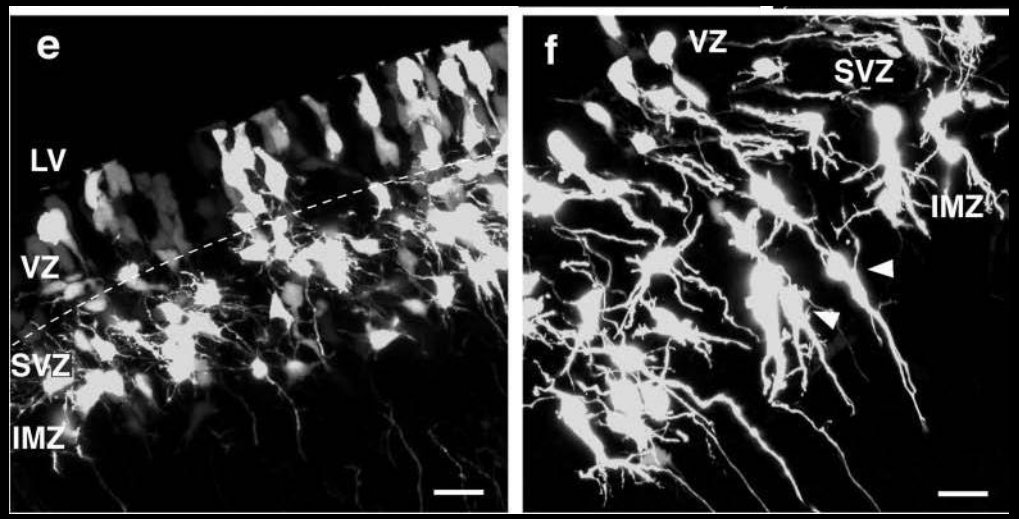

E14-E18

Nestin red, migrating cells EGFP

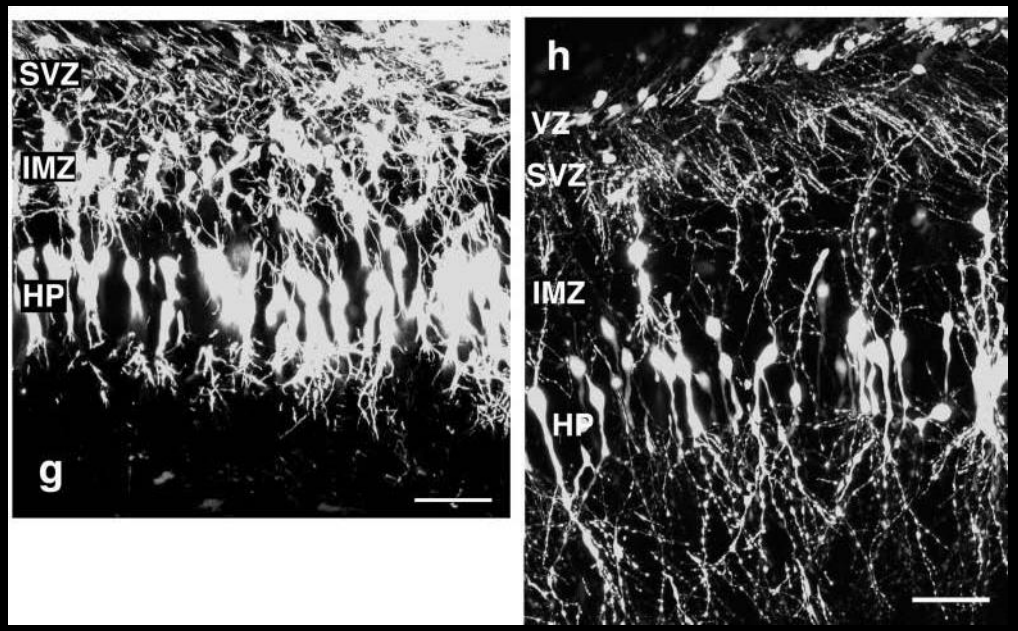
Nakahira and Yuasa, 2005

# CA1 cell migration (visualized fluorescently)

Cells are first multipolar. Then bipolar, e.g. 4 days to reach the pyr. layer




E14-E16 E14-E17



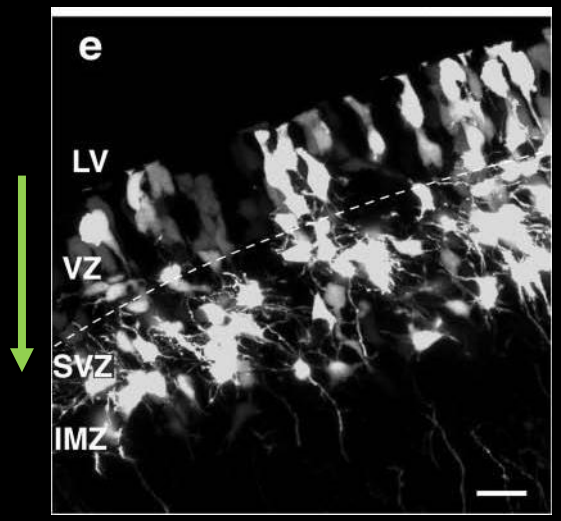

E14-E18 E14-P2

Slow migration rate

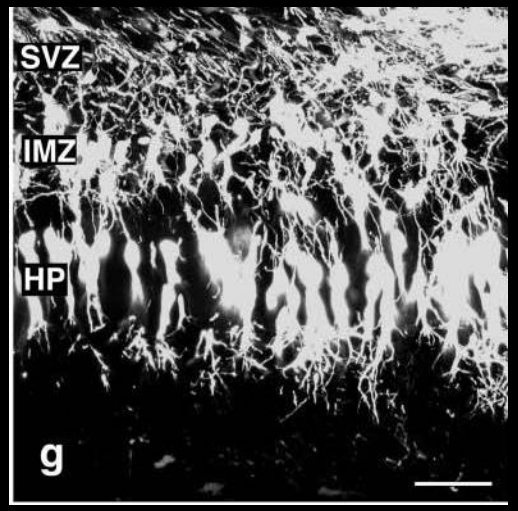
Nakahira and Yuasa, 2005

# Slower migration than neocortex

Hippocampus

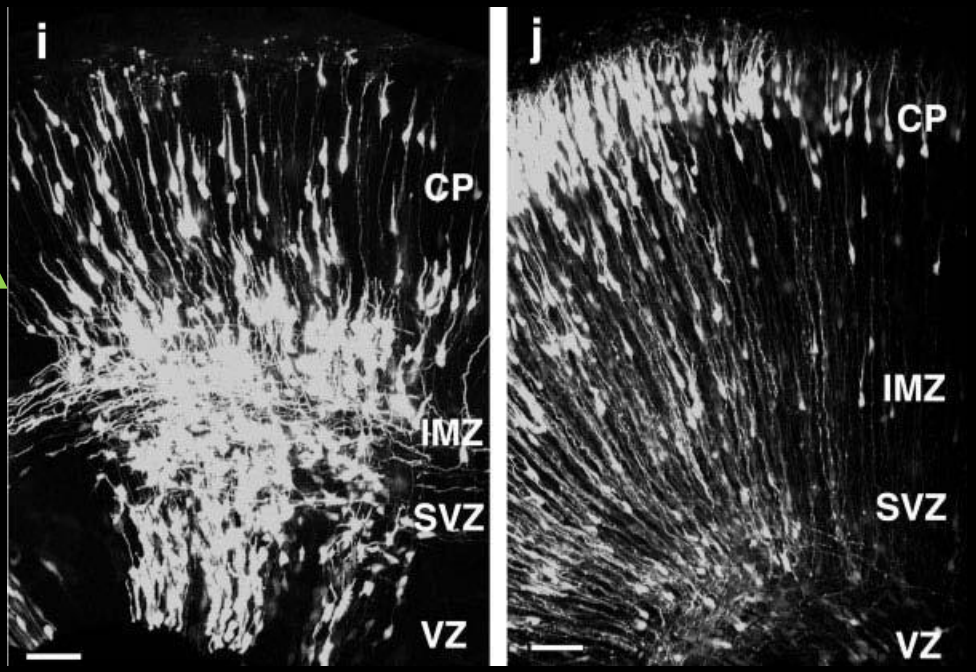



E14-E16




E14-E18

Neocortex



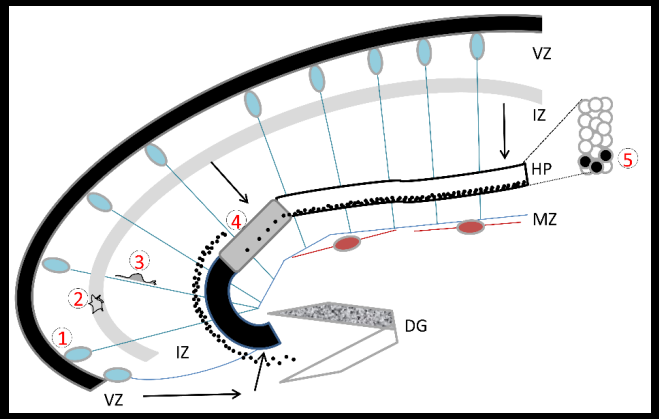

E14-E16

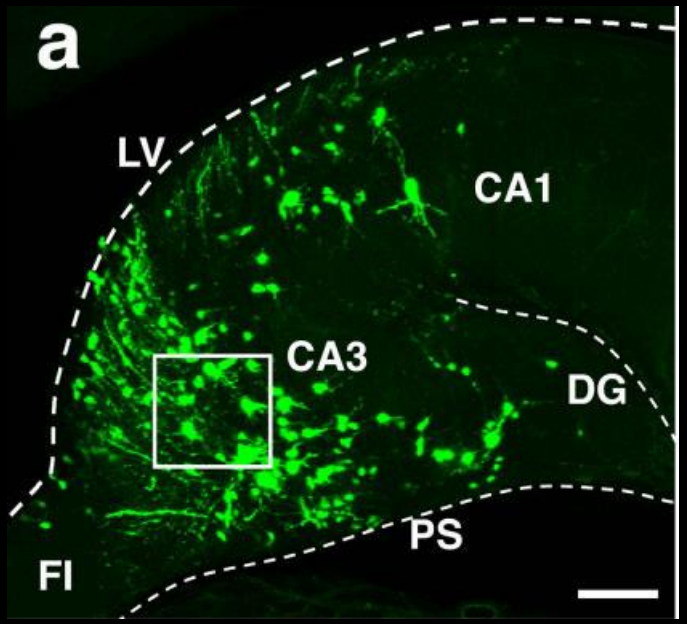
E14-E18

Slow multipolar to bipolar transition?
Awaiting afferents? Coordination with other cell types?

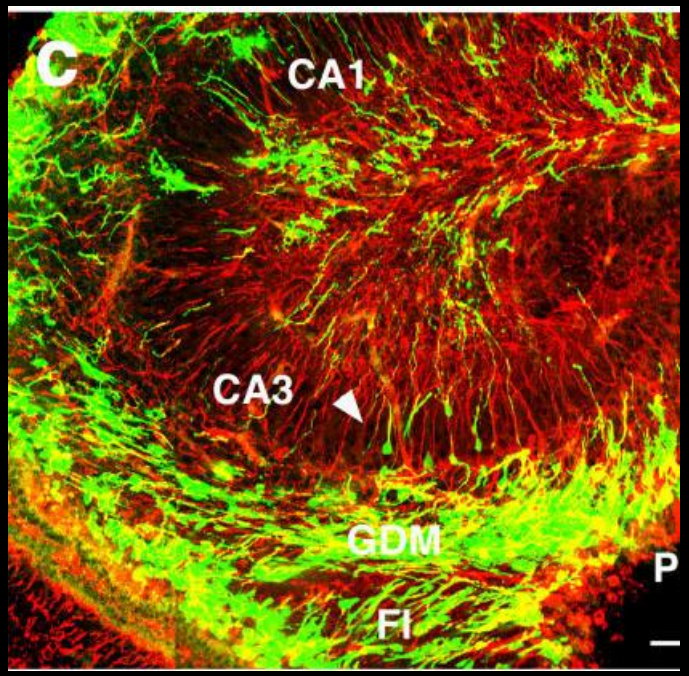
Nakahira and Yuasa, 2005

# CA3 migration




E14-E16



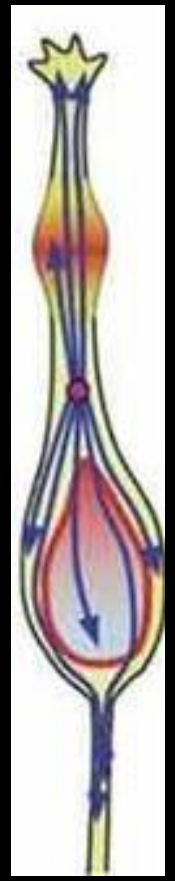

E16-E18

Majority of cells born at E14

Later born cells (E16), migrate tangentially before migrating radially

Nakahira and Yuasa, 2005

# Summary 3: CA cell migration



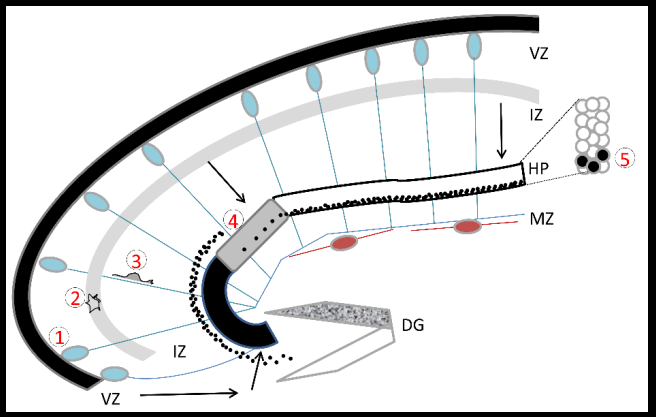
CA cells migrate in close apposition with radial glia cell processes

Multipolar, then bipolar morphologies

Much slower migration compared to neocortical neurons

CA3 pyramidal cells longer curved route to arrive in pyramidal cell layer

Various gene mutations affect this migration process
(Dcx, Lis1, Kif2a, Tuba1a function at microtubule cytoskeleton)

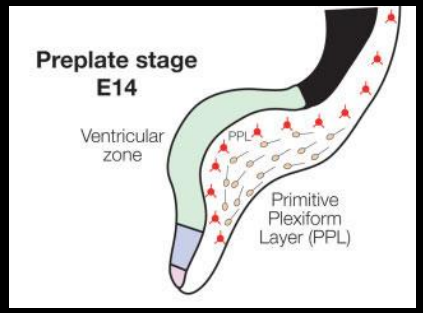


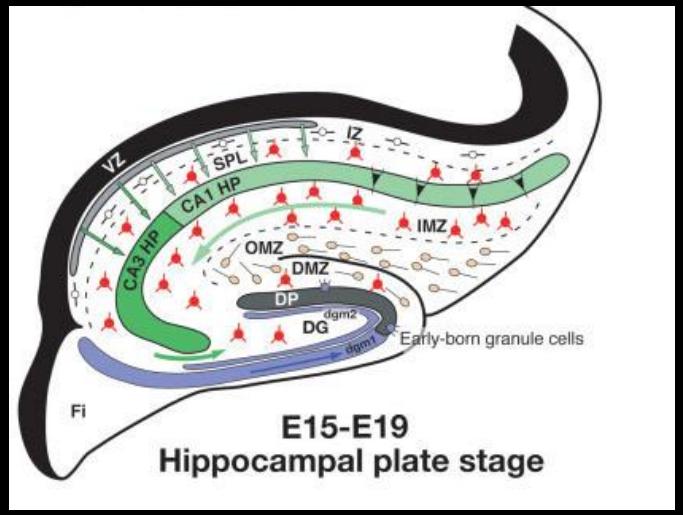
# Mouse hippocampal development

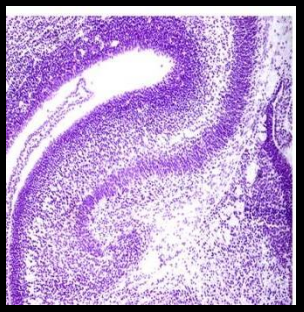
Adult

The origins of the hippocampus (medial telencephalon, hem)
Initial cell amplification, pioneer neurons (E8.5-10.5)

Neurogenesis, migration and final destination
- CA field (E11-E16)
Dentate gyrus granule cells (E11-postnatal)
Interneurons (E10-E14)



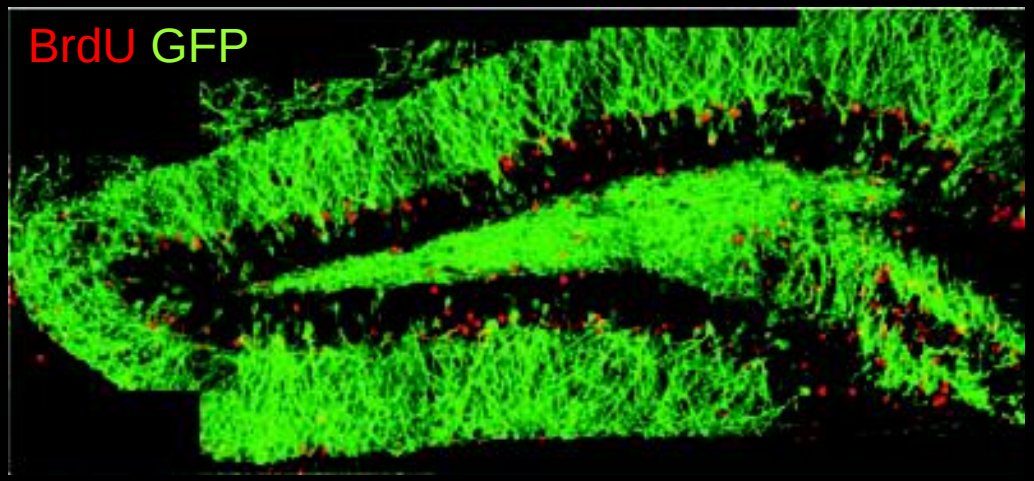



*Danglot et al., 2006*

# Neurogenesis – DG cells

3H thymidine labeling showed that many granule cells born at E16 (Angevine 1965)

However, some granule cells are also produced early



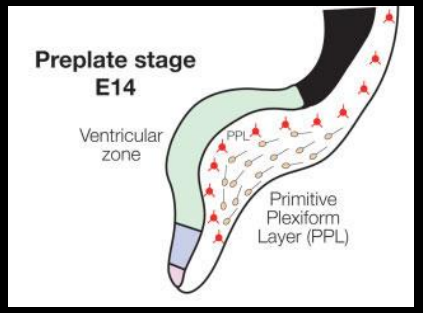

BrdU E11.5, analysis P30

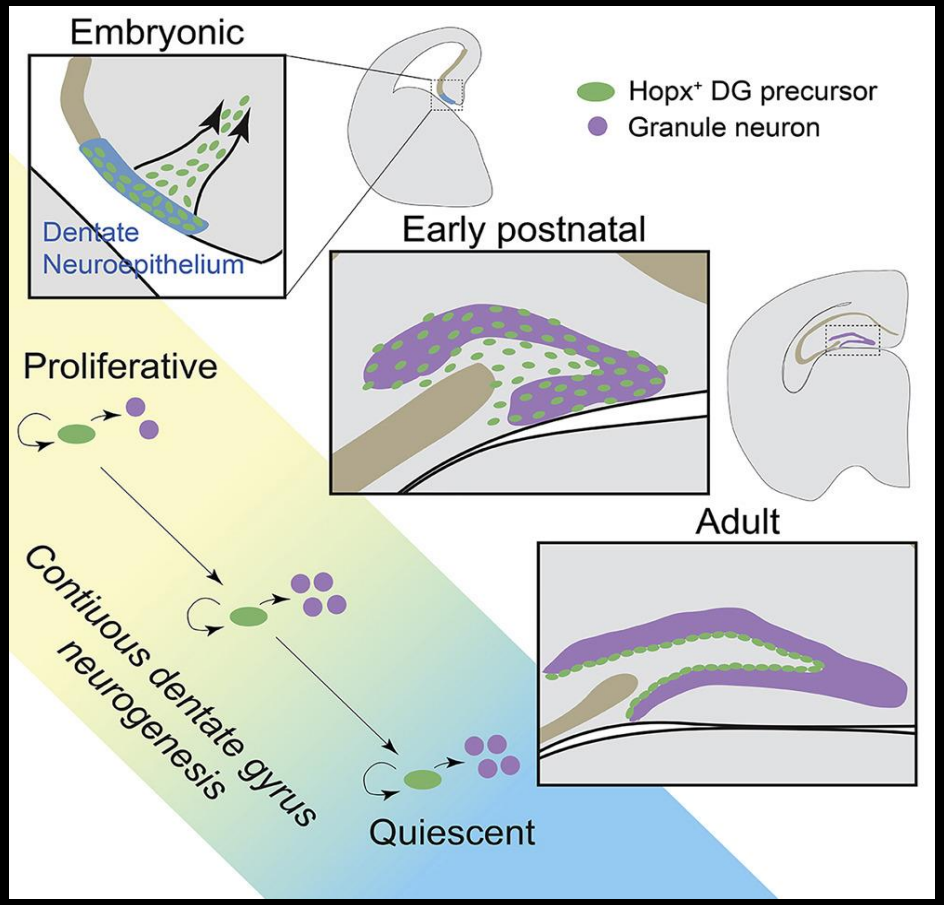
Formation of synapses between neurons (GC and CA3) born in the same temporal window?

Selective connectivity between temporally matched subpopulations of cells?

Deguchi et al., 2011

# A Common Embryonic Origin of Stem Cells Drives Developmental and Adult Neurogenesis

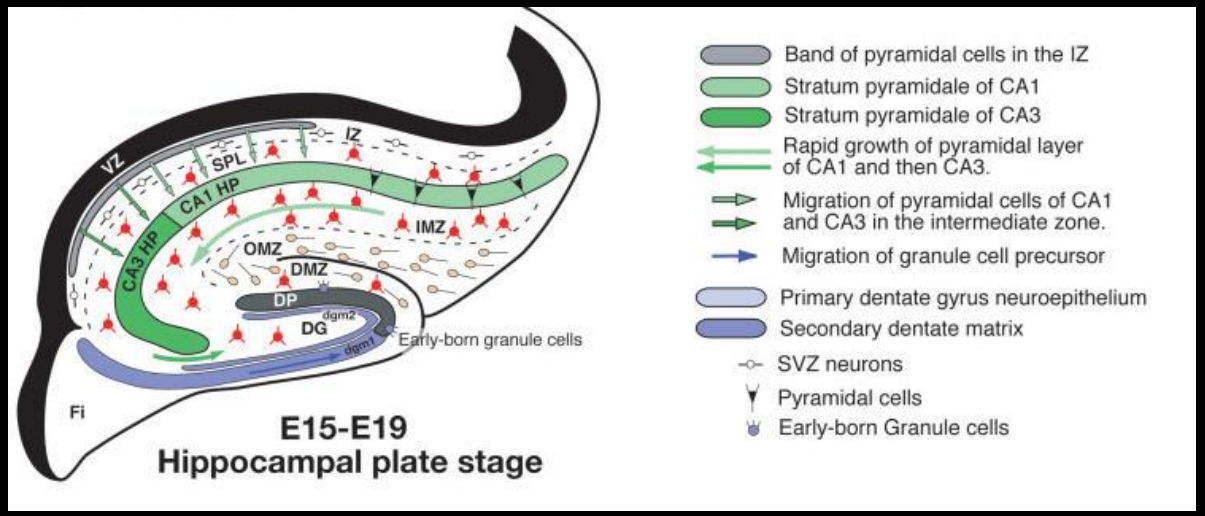


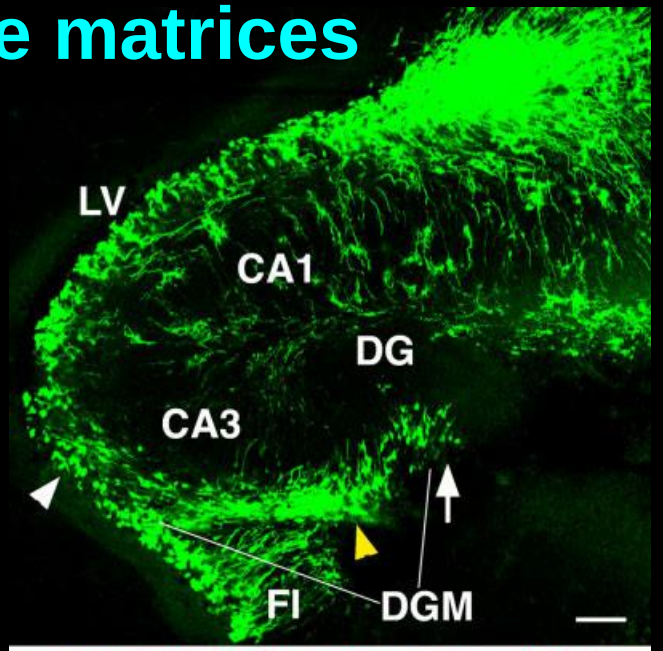



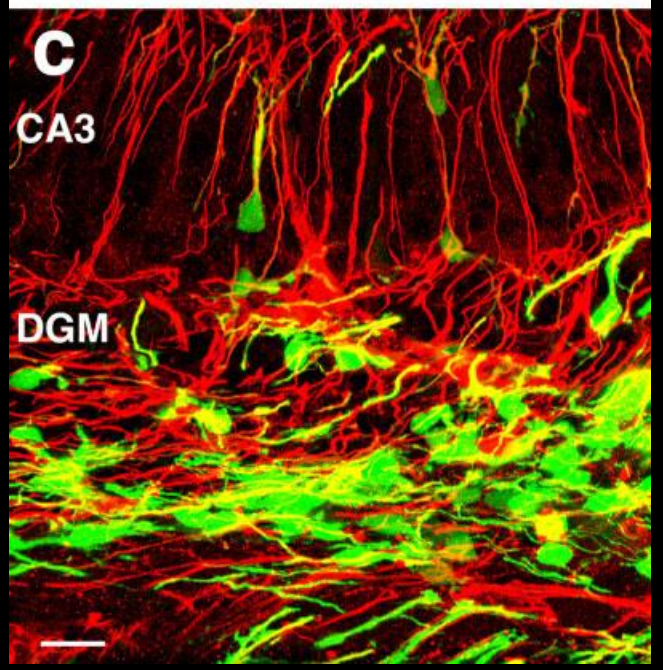

Proliferating cells migrate

*Berg et al., 2019, Cell*

# Tangential migration to form dentate matrices




Nakahira and Yuasa, 2005

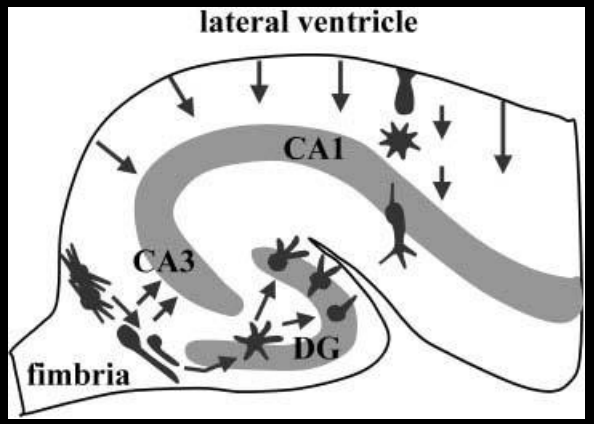
Waves of neurogenesis (E16)

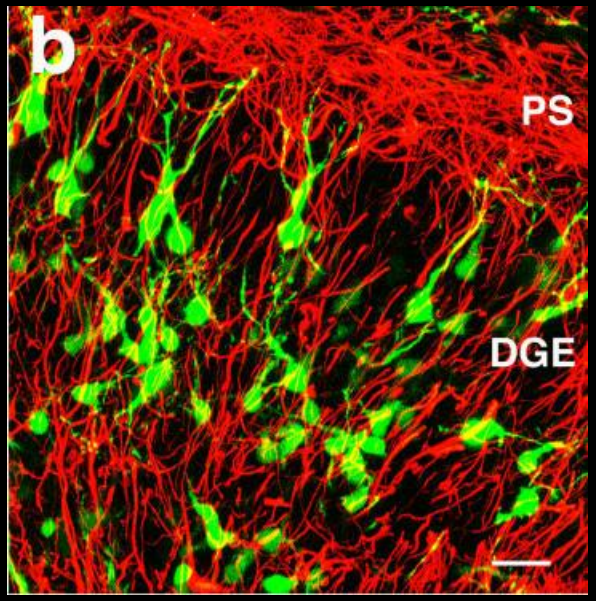
Tangential migration of neurons and precursor cells through subpial layer, by E18 cells become obvious in the DG region

E16-E18

*Danglot et al., 2006; Hatami et al, 2018*

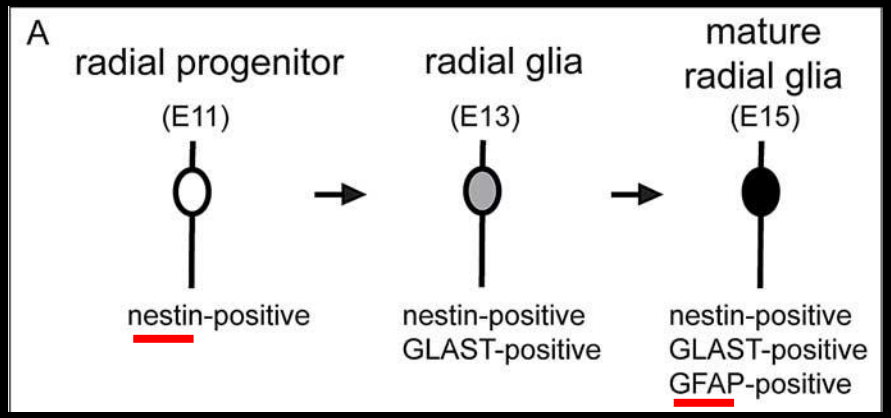
# Radial migration within the dentate gyrus

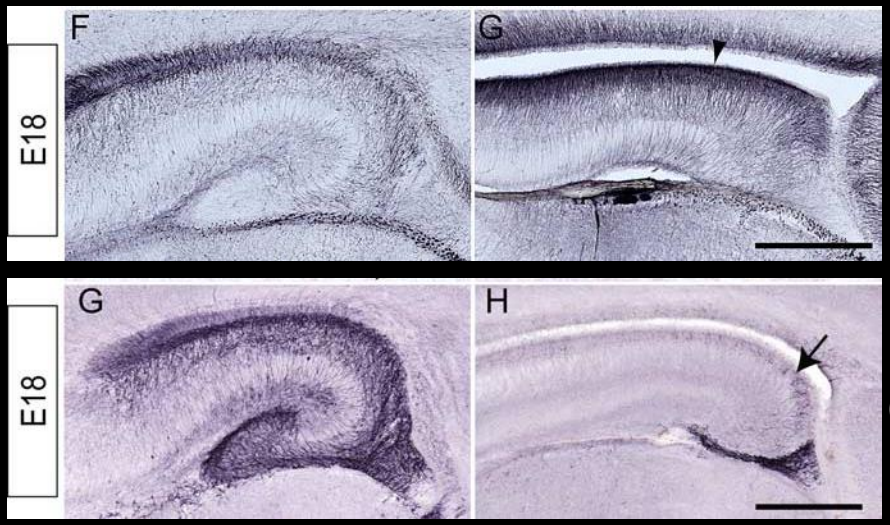


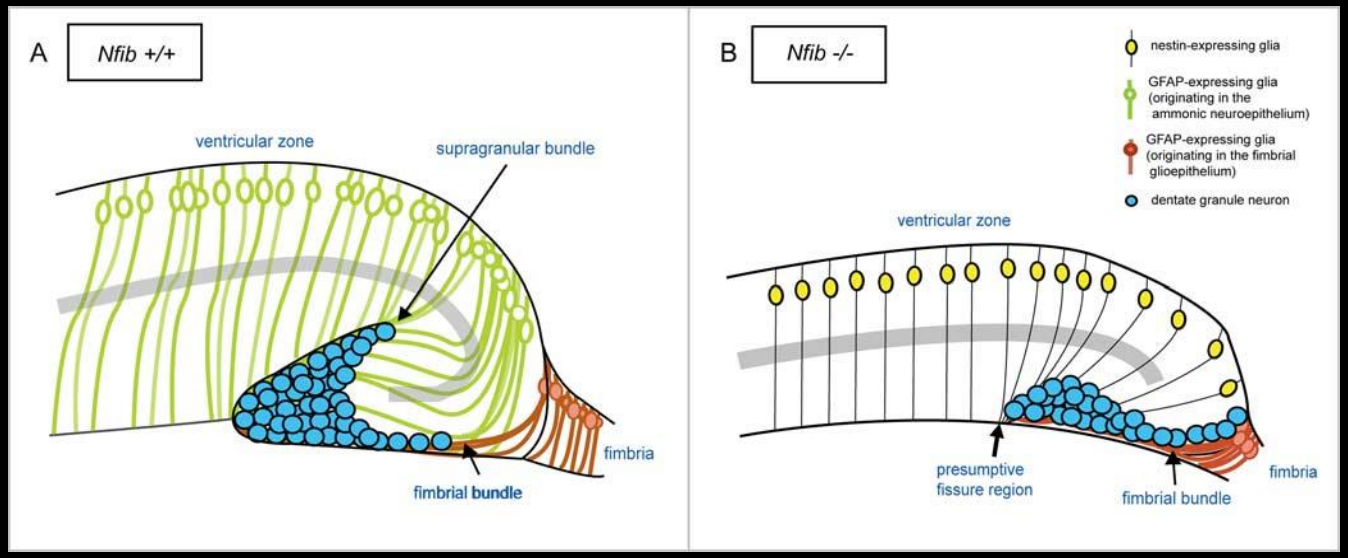




E16-P2

# *Nuclear factor 1b* mutants – abnormal RG cells



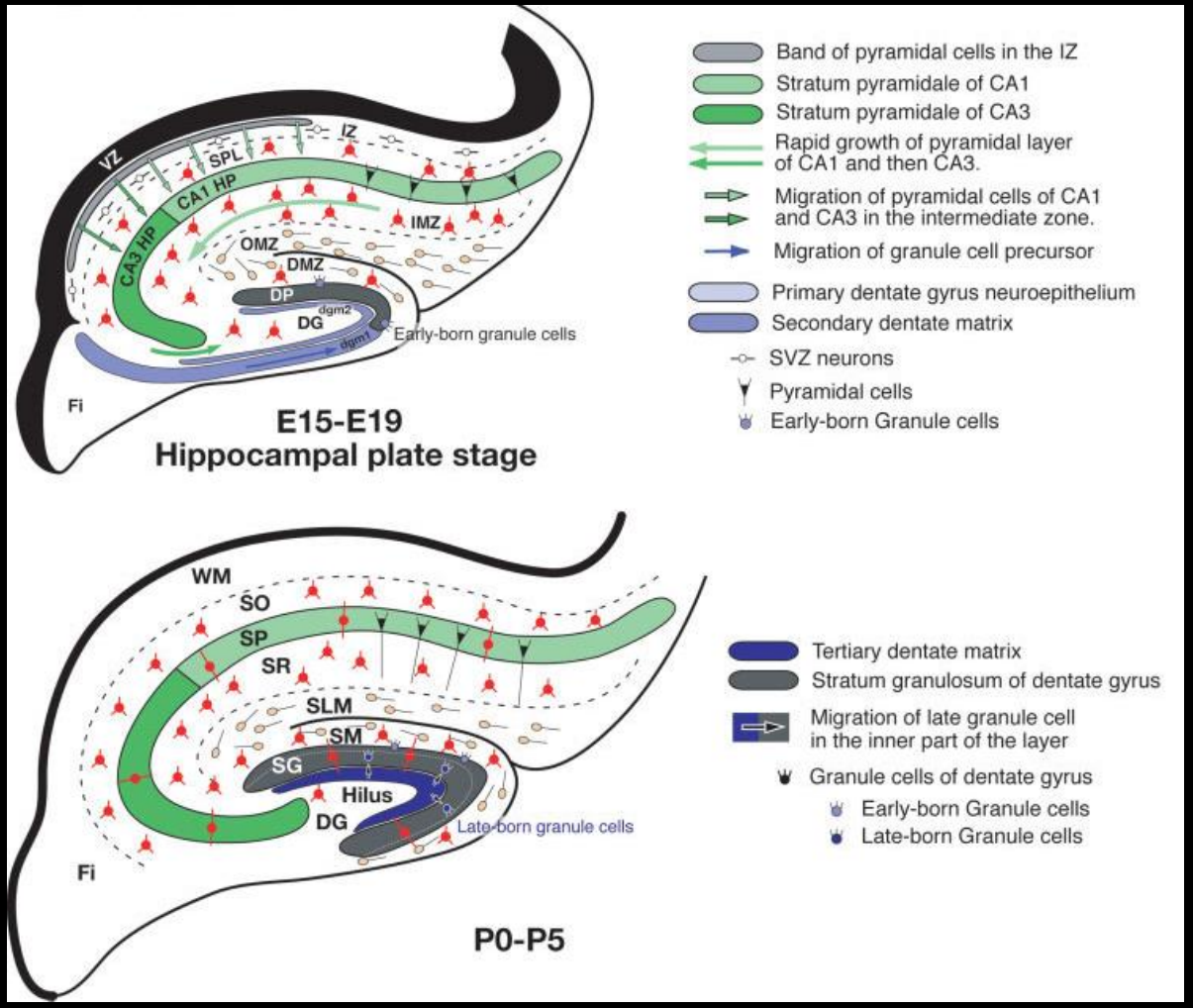




Barry et al., 2008

DG cells unable to leave subpial migratory stream

# Tertiary matrix and adult neurogenesis




Altman and Bayer, 1990; reviewed by Danglot et al., 2006; Berg et al., 2019

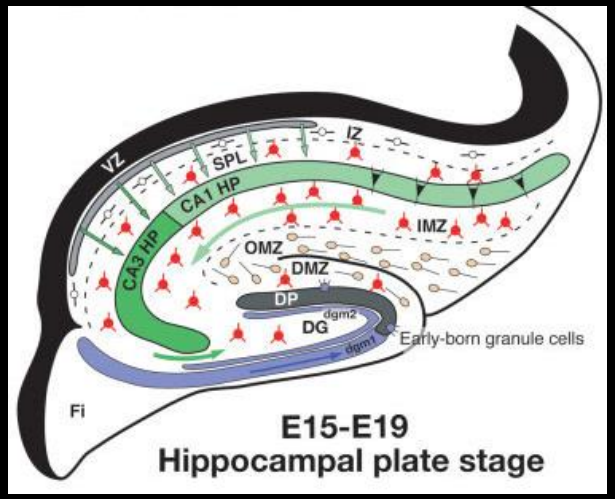
# Summary 4 – development of the DG

Primary, secondary and tertiary dentate matrices

Subpial migratory stream (contains progenitors and post-mitotic neurons)

Supragranular blade develops before infragranular blade

RG-like cells in DG region also important for DG development
(formation, maturity, maintenance)



# Mouse hippocampal development

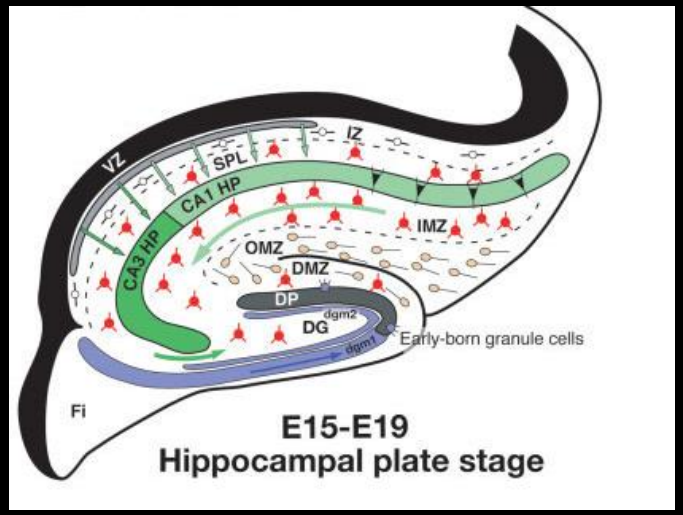
Adult

The origins of the hippocampus (medial telencephalon, hem) progenitors and pioneer neurons (E8.5-10.5)

Neurogenesis, migration and final destination

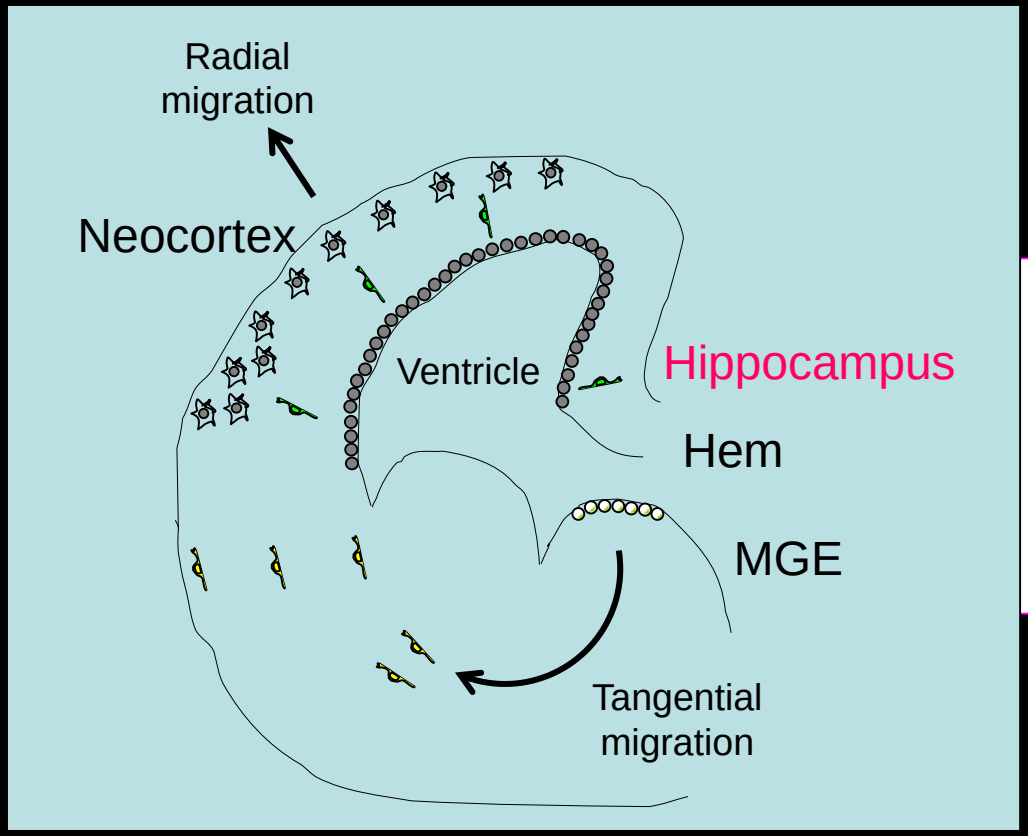
- CA field (E11-E16)
- Dentate gyrus granule cells (E11-postnatal)

Interneurons (E10-E14)



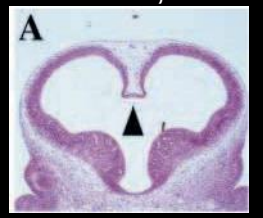

*Danglot et al., 2006*

# Neurogenesis, migration




VZ, ventricular zone
IZ, intermediate zone
CP, cortical plate

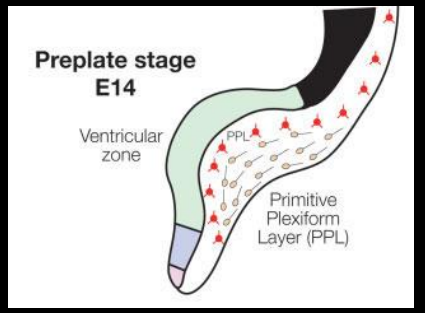
E12,5




1. Proliferation
2. The generation of neurons
3. Migration
4. Growth of axons and dendrites
5. The formation of synaptic connections

Ganglionic eminences : ventral telencephalon

# Early generated pioneer GABA neurons




Very few in hippocampus (eg 5 cells per section)

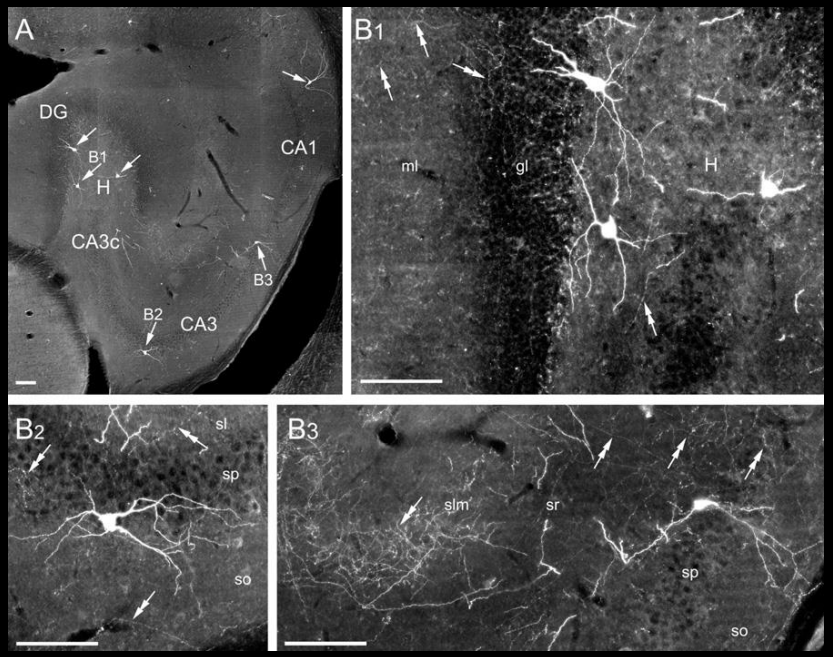
But very extensive axonal arborisation

GABA projection neurons

High connectivity

Functional ‘hubs’

Important for developing hippocampal network and persist into adulthood



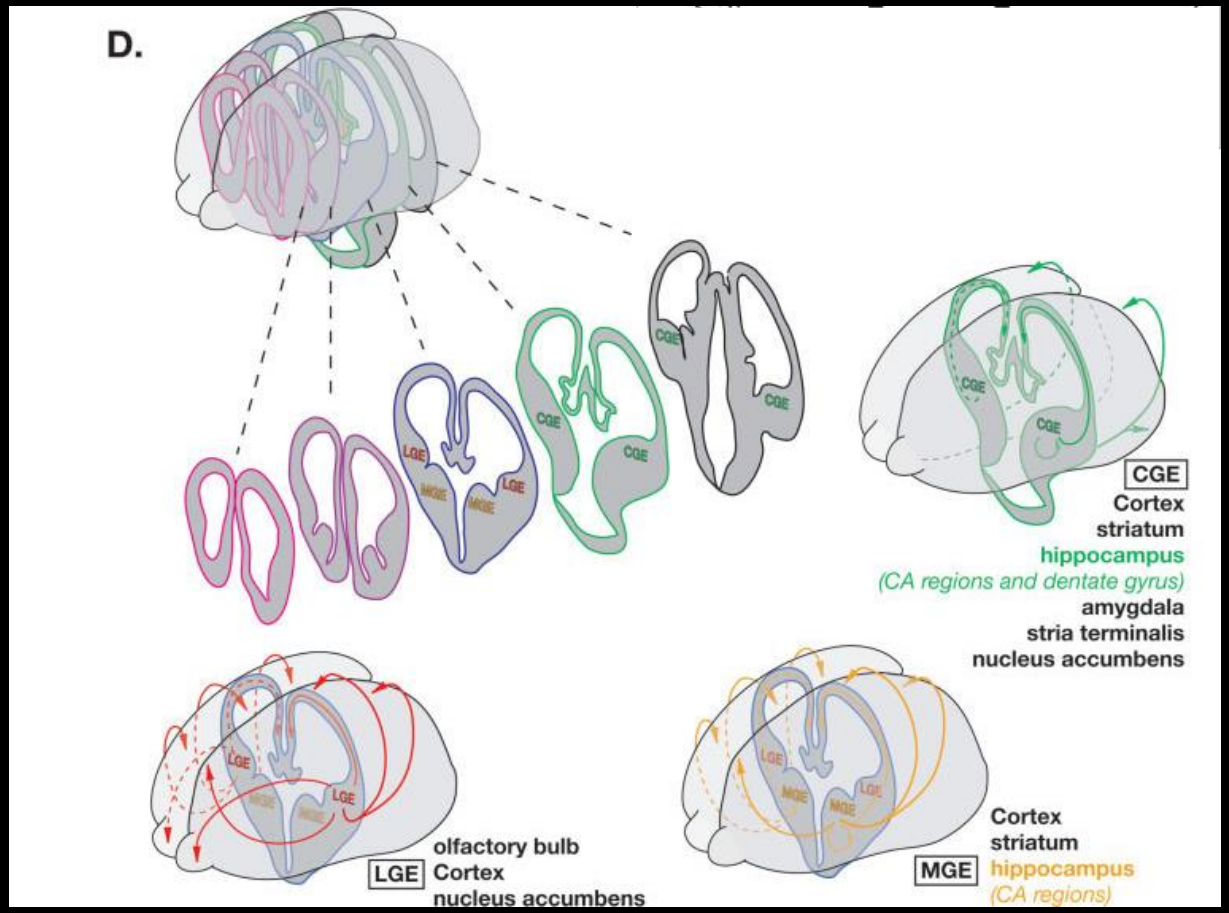

Dlx1/2 Cre ERT2 / reporter line
Tamoxifen E9.5 –E10

Picardo et al, 2011

# Hippocampal INs derived from MGE and CGE

CA: E12-13

DG: E13-14



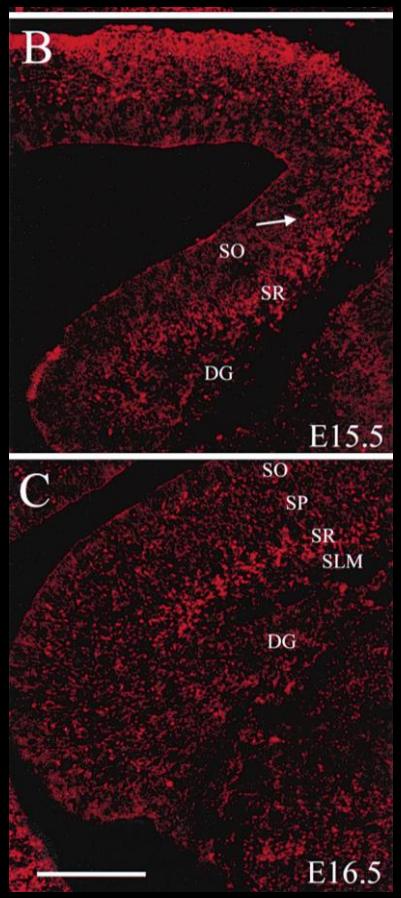

CCK, calretinin, VIP…

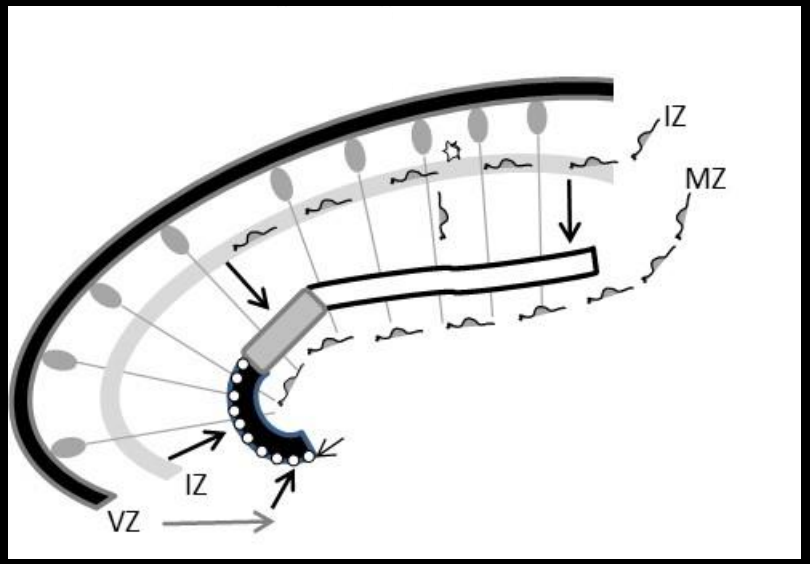
PV, SST, NOS..

Different IN subtypes produced in different locations and with different times of genesis

Danglot et al., 2006; Tricoire et al., 2011; Pleasure et al., 2000; Wichterle et al., 2001; Polleux et al., 2002; Nery et al., 2002

# Streams of GE-derived hippocampal INs

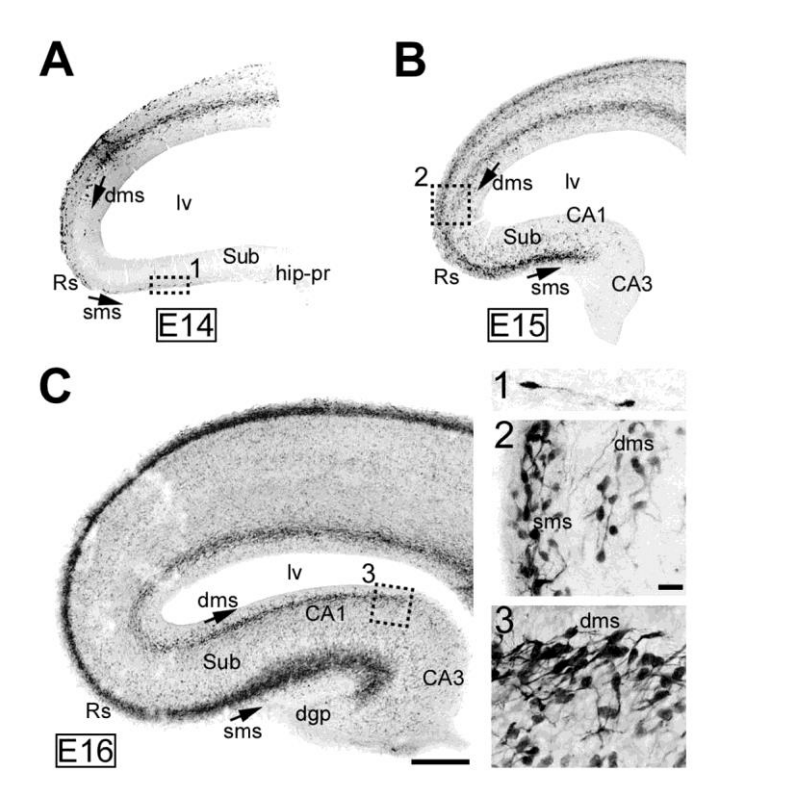



Dlx2+ IN streams visible in HC from E15.5

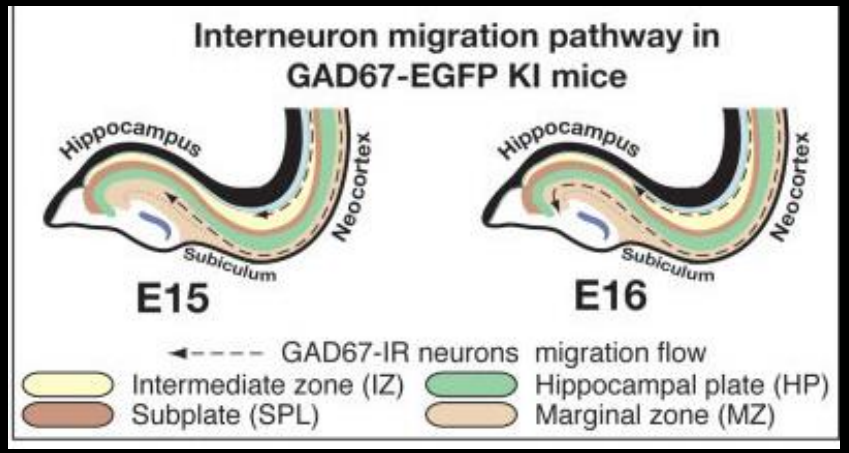
*Soriano et al., 1989a,b; Pleasure et al., 2000*

# GAD67-EGFP mouse - Two major migrating streams




Superficial (MZ/SR) and deep (SVZ/SO) migratory streams

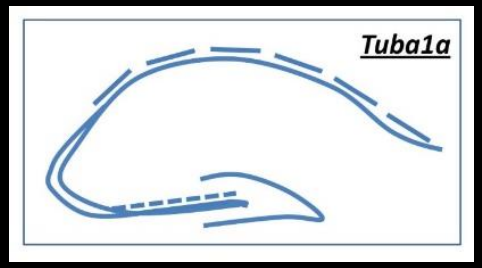
Superficial stream contributes the most INs, goes as far as CA3 region
E16: deep stream at CA1/CA3 junction

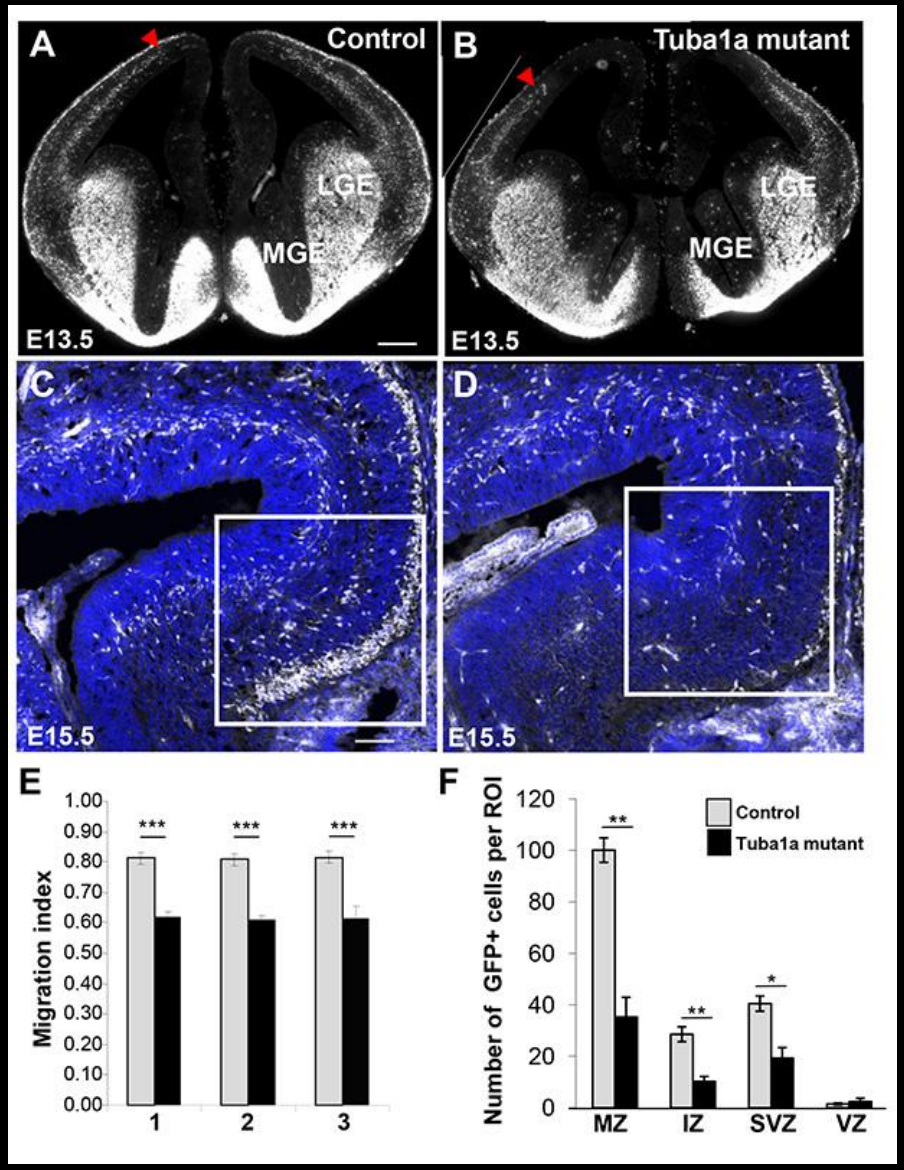



Both MGE and CGE contribute INs to both streams

Manent et al., 2006; Danglot et al., 2006

# IN migration defects in Tuba1a mutants

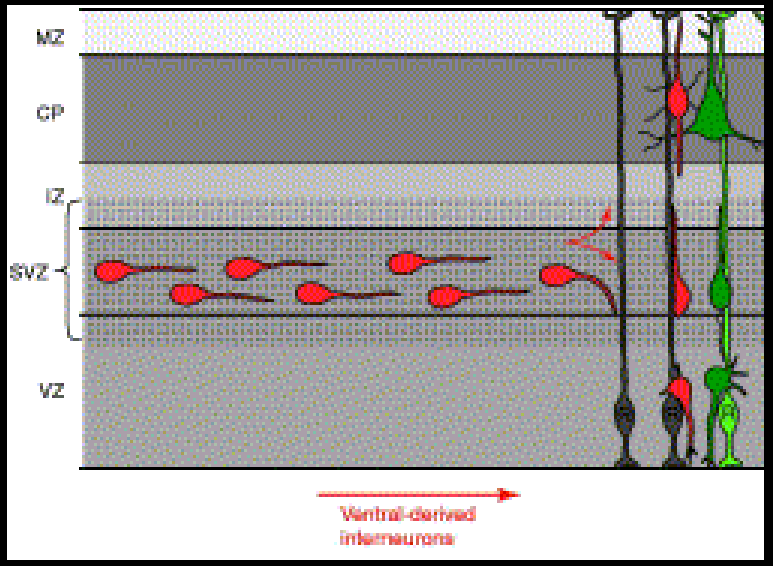


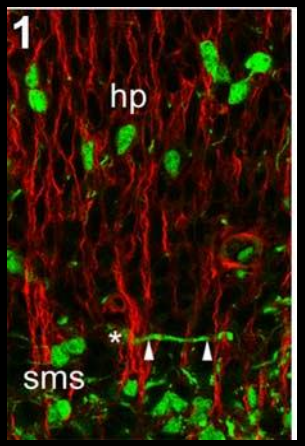




*Belvindrah et al 2017*

# Final positioning – tangential to radial migration

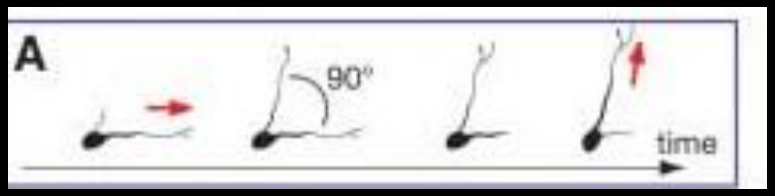
Leading process of interneurons initially perpendicular to RG extensions




Hippocampal
plate invasion
E16/E17 onwards

Then parallel (radial migration)




Polleux et al., 2002; rev by Marin 2013

# Summary 5 - Interneurons

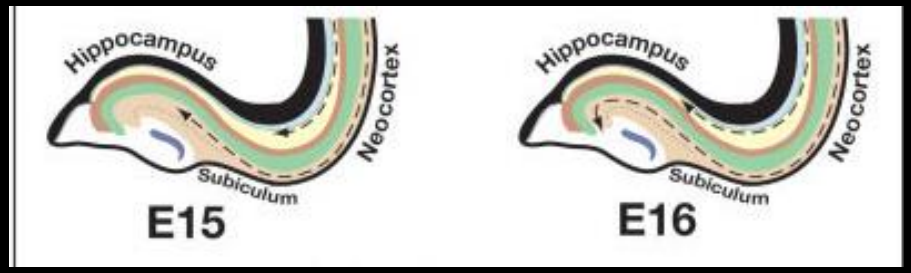
Pioneer GE GABAergic projection neurons (before E10.5)

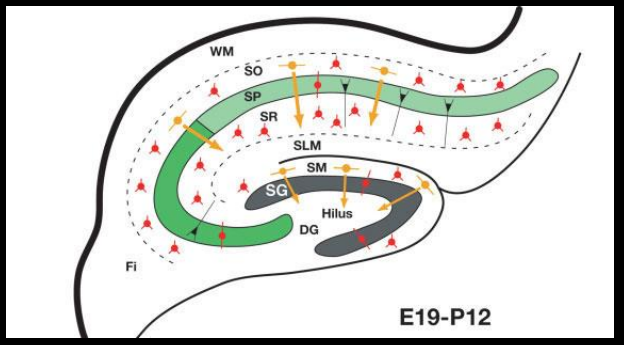
Majority of INs generated in the GEs (MGE and CGE) between E12-E14

Long migration pathway, arrive in hippocampus from E15

Tangential then radial migration (E16 onwards)

Final positioning peri- and postnatally

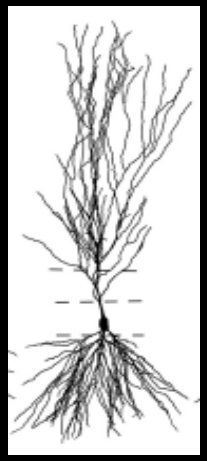



Danglot et al., 2006, Morozov et al., 2006

# The next developmental steps……

(late prenatal – postnatal)



apical

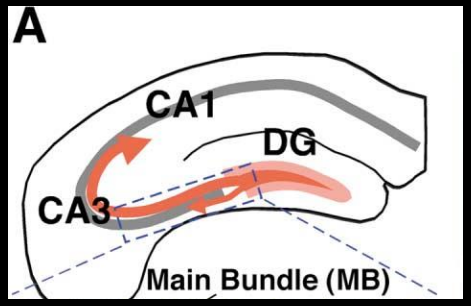
basal

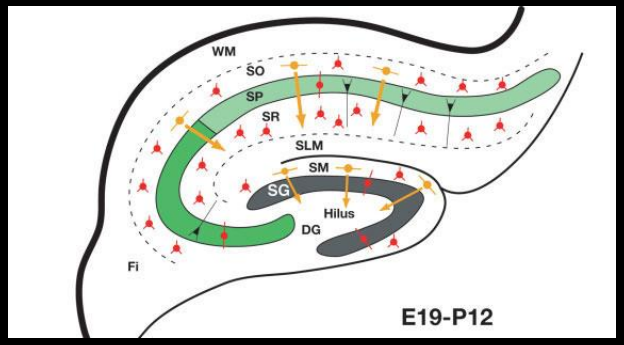
Axo- and dendrito-genesis

Targeting

Synaptogenesis

Refinement




Cells progressively acquire mature characteristics in the postnatal period

# Axogenesis, synaptogenesis and pruning

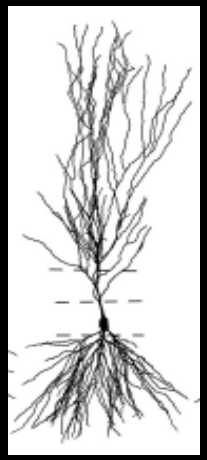
Axon with growth cone is guided to its target by positive and negative cues (netrin, slit, semaphorin, ephrin…)

Cell adhesion molecules seem critical for correct targeting and synapse formation

Many connections are transient requiring pruning / some cell death also occurs

Final subtle readjustments to create terminal arborization (final pattern of synaptic contacts)

# GC mossy fibers connect to CA3 cell dendrites




Adhesion

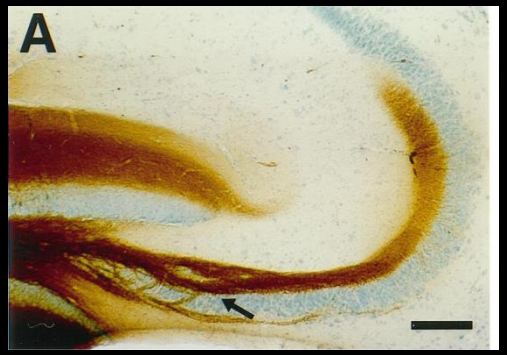
Nectin, Chl1, cadherin, Ncam-180

Axon guidance

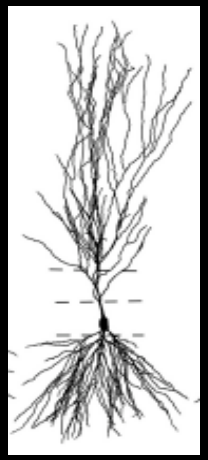
Plexin A2, Plexin A4

Plexin A3, Neuropilin 2

Ephrin B3….

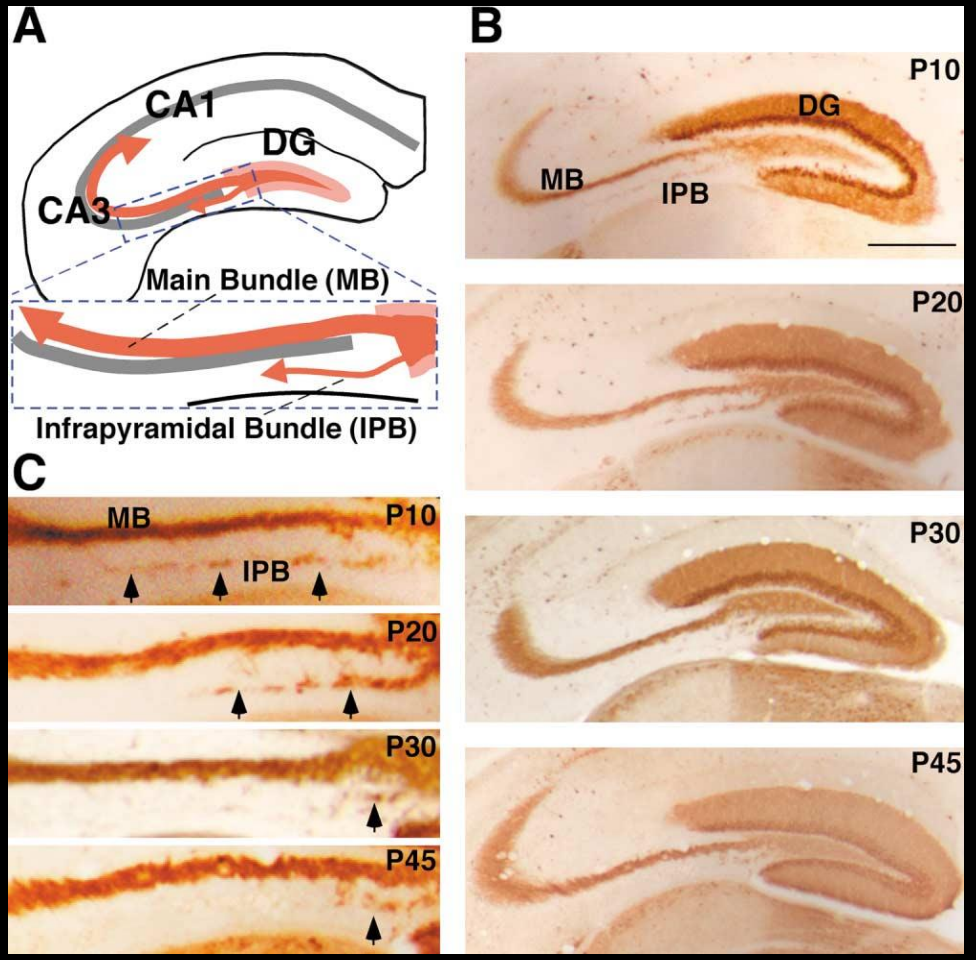


# Pruning between P10-P45



apical

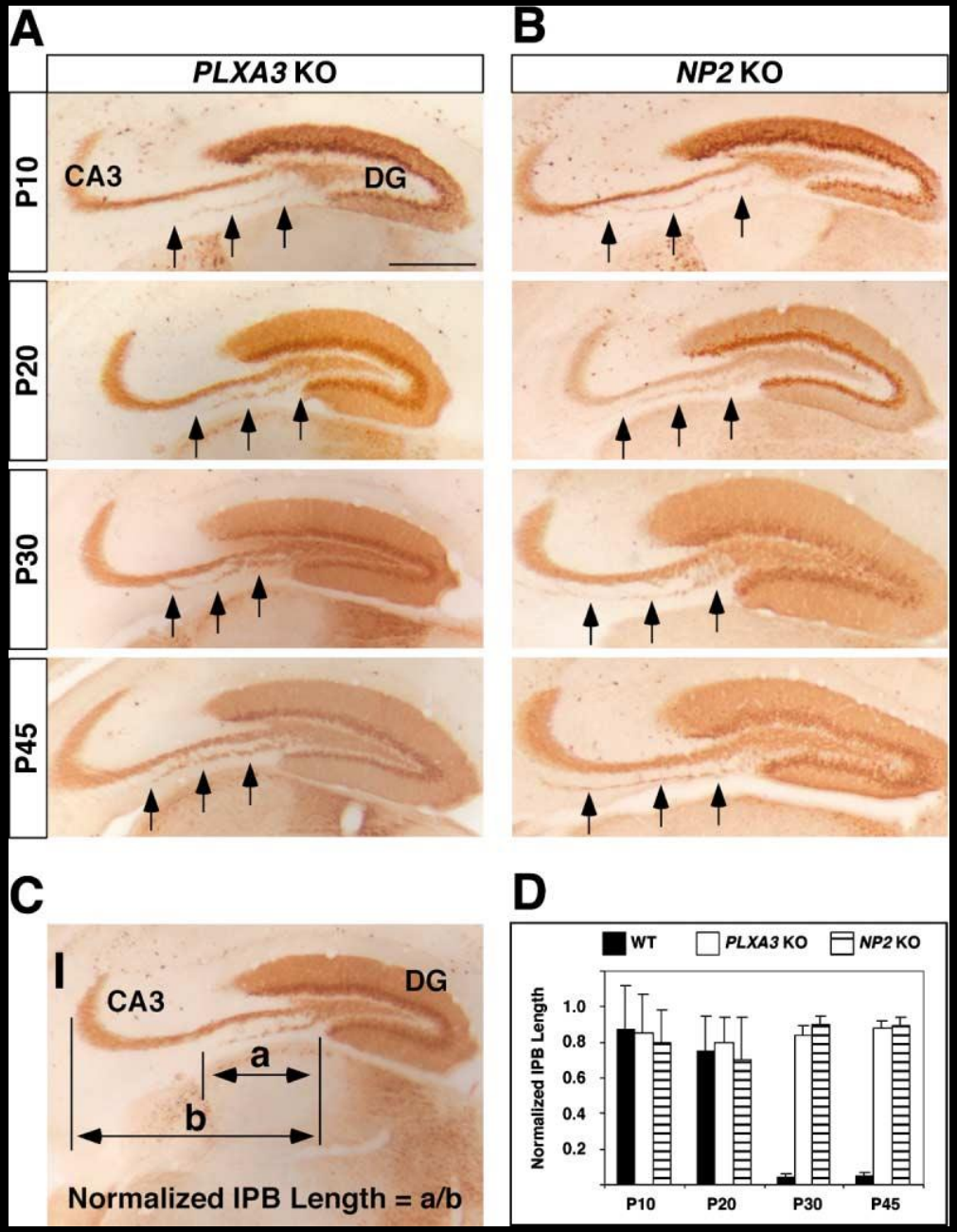
basal




Bagri et al., 2003

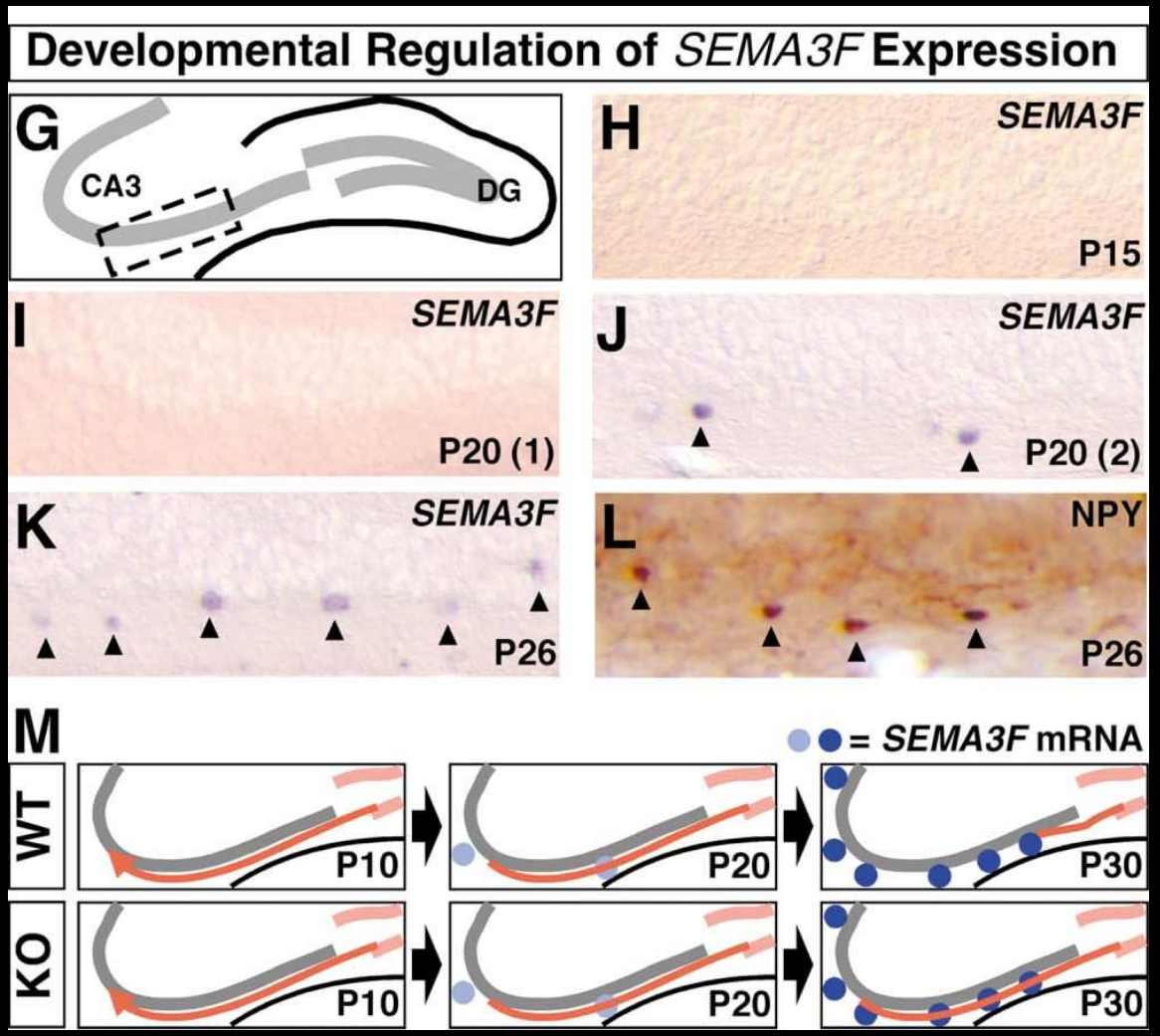
Mutants:
Plexin A3
Neuropilin 2

Basal dendrite connections not pruned




Bagri et al., 2003

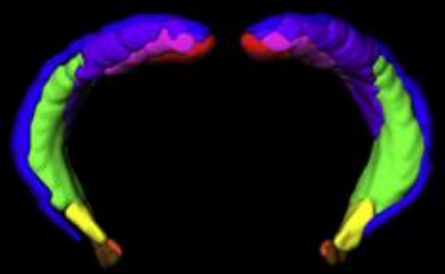
# Semaphorin 3



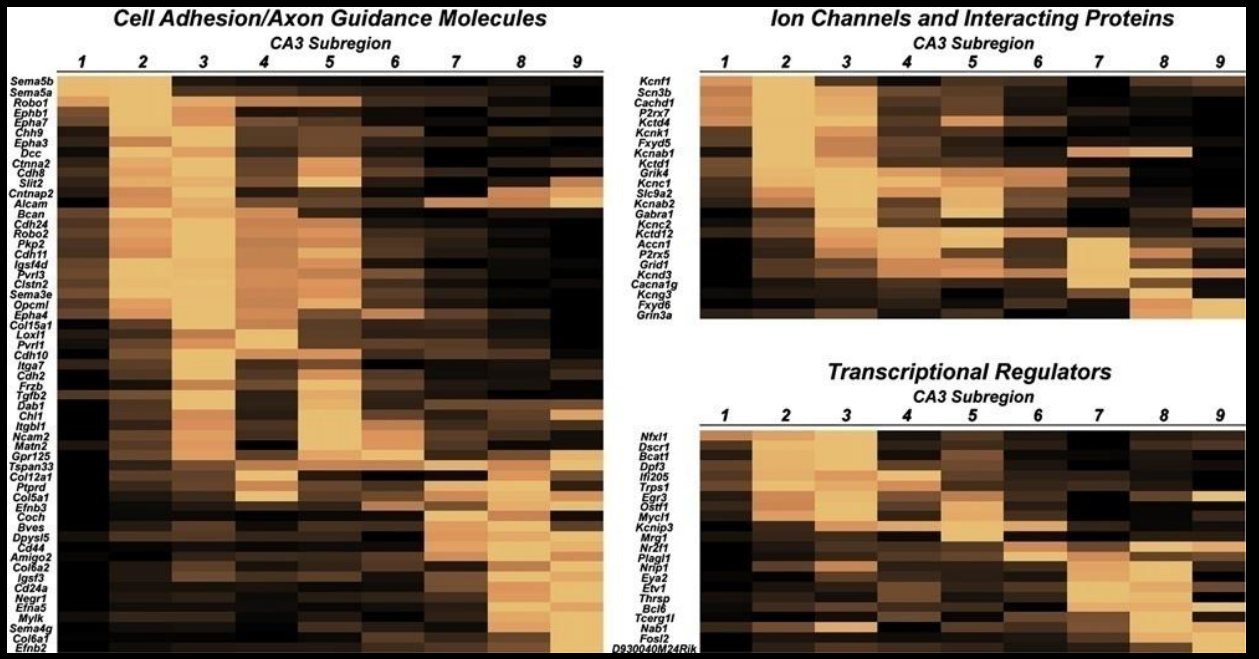

Plex mutant axons do not respond to Sema 3F

# Connectivity and cell surface molecules

Signals on pre and post-synaptic cells trigger synaptogenesis and allow segregation of terminals.



Differentially expressed genes in the CA3 region




Molecular patterns seem to correlate with anatomical borders identified by tracing studies

Establishment and maintenance of topographic specificity

Different cell compartments, different signals.

Thompson et al., 2008

# Axogenesis, synaptogenesis and pruning

Axon with growth cone is guided to its target by positive and negative cues (netrin, slit, semaphorin, ephrin…)

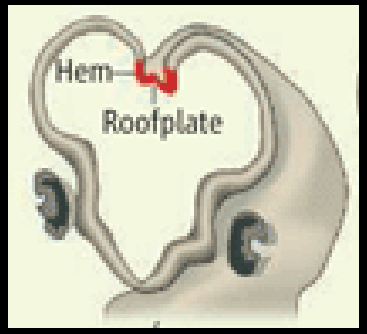
Cell adhesion molecules seem critical for  correct targeting and synapse formation

Many connections are transient requiring pruning / some cell death also occurs

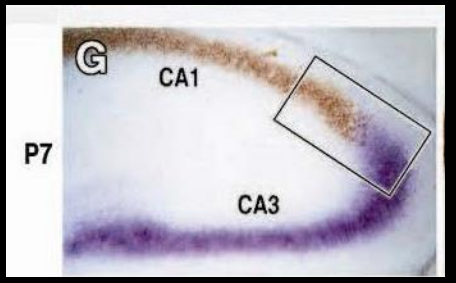
Final subtle readjustments to create terminal arborization (final pattern of synaptic contacts)

Many factors involved!

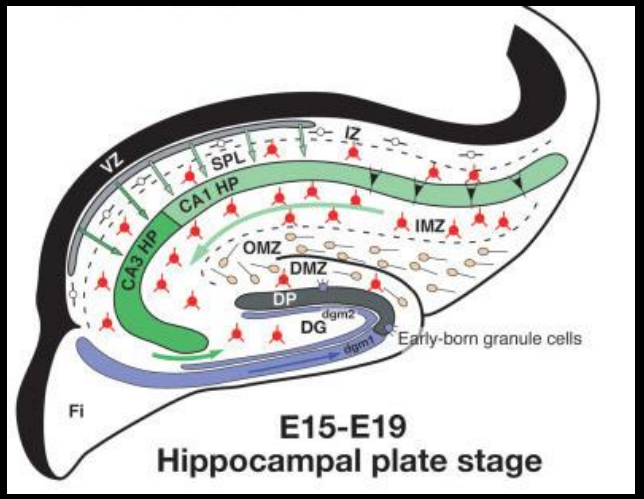
# Overall summary



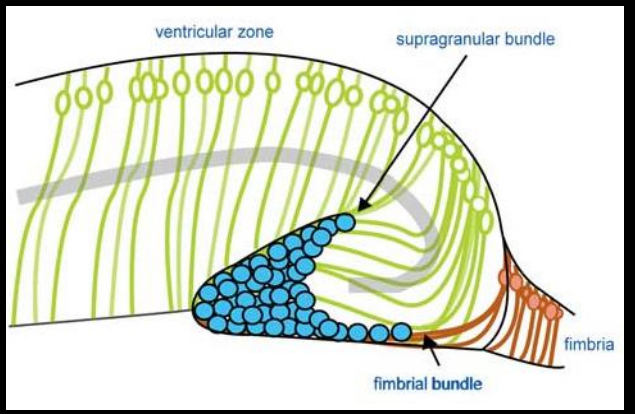

E8.5 - organizer




CA1/CA3 neurogenesis



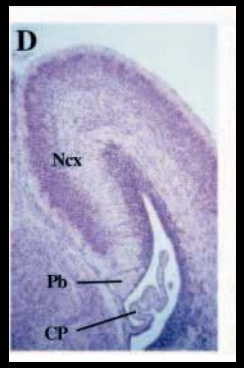

Migration

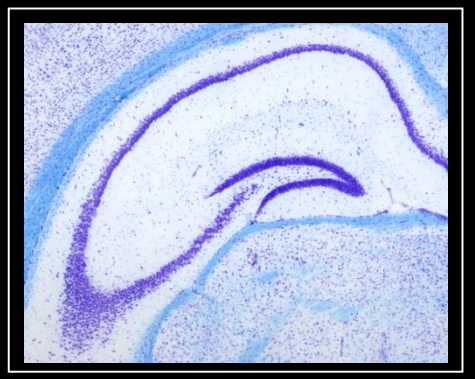



RG cells

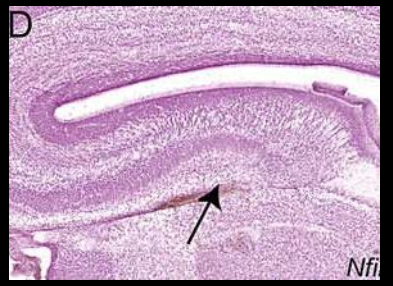
# Hippocampal malformations

Hem
Wnt3a

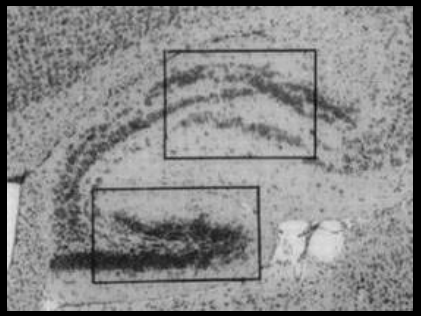


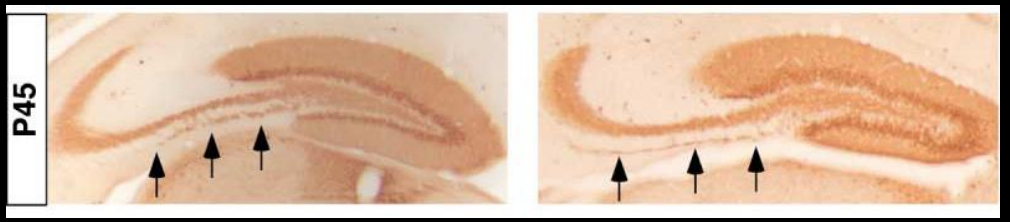
DG
Nf1b



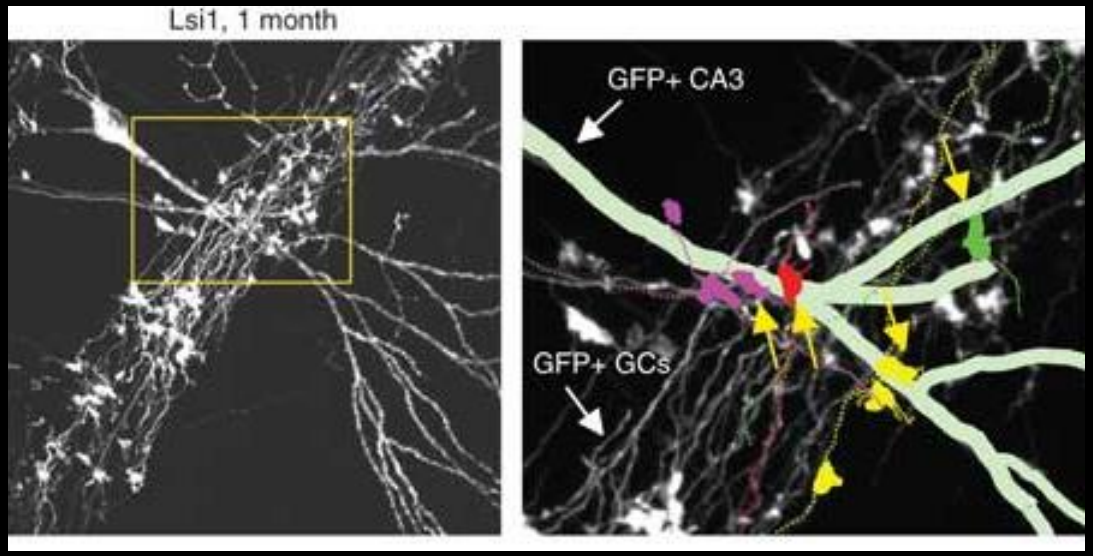

CA (and IN) migration
Dcx
Lis1
Tuba1a



Pruning
Plexin A3



# Hippocampal microcircuits



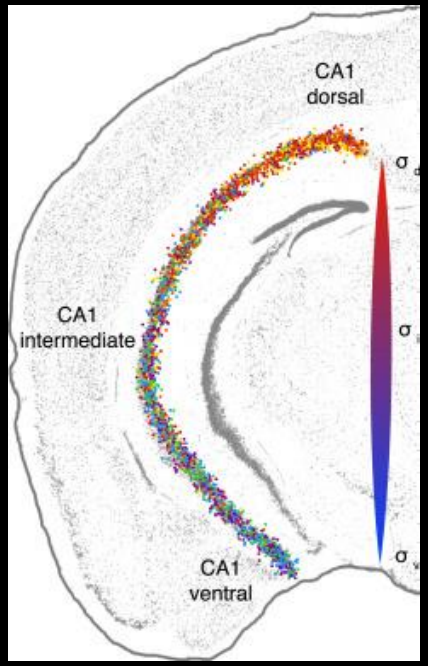

At least 65% of the mossy-fiber inputs to labeled CA3 cells came from labeled granule cells

Birth timing could be important for connectivity

Deguchi et al., 2011

# Gradients of Gene Expression in Area CA1

Cell diversity

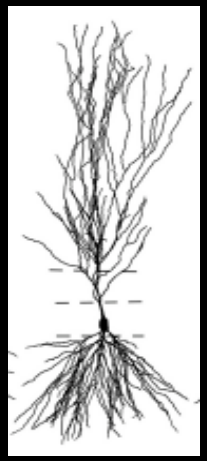



Radial
Septo-temporal….

*Cembrowski et al, 2016; Tushev and Schuman, 2016*

# The next developmental steps……

(late prenatal – postnatal)



apical

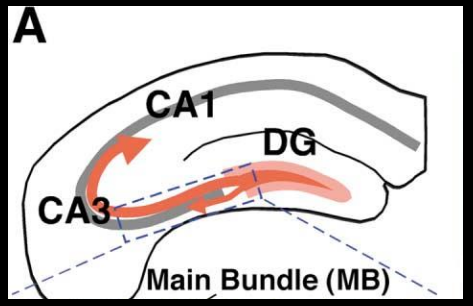
basal

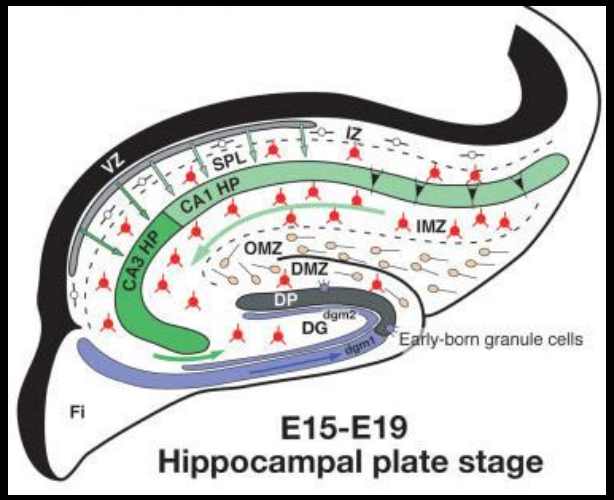
Axo- and dendrito-genesis

Targeting

Synaptogenesis

Refinement




Cells progressively acquire mature morphological characteristics in the postnatal period (first three postnatal weeks)