In vivo clonal analysis for cell lineage in neurodevelopmental studies

Lineage tracing by clonal analysis

- Consists in **quantitative analyses** of clones originated from a single stem/progenitor cell

- Allows for **specific labeling** of single stem/progenitors

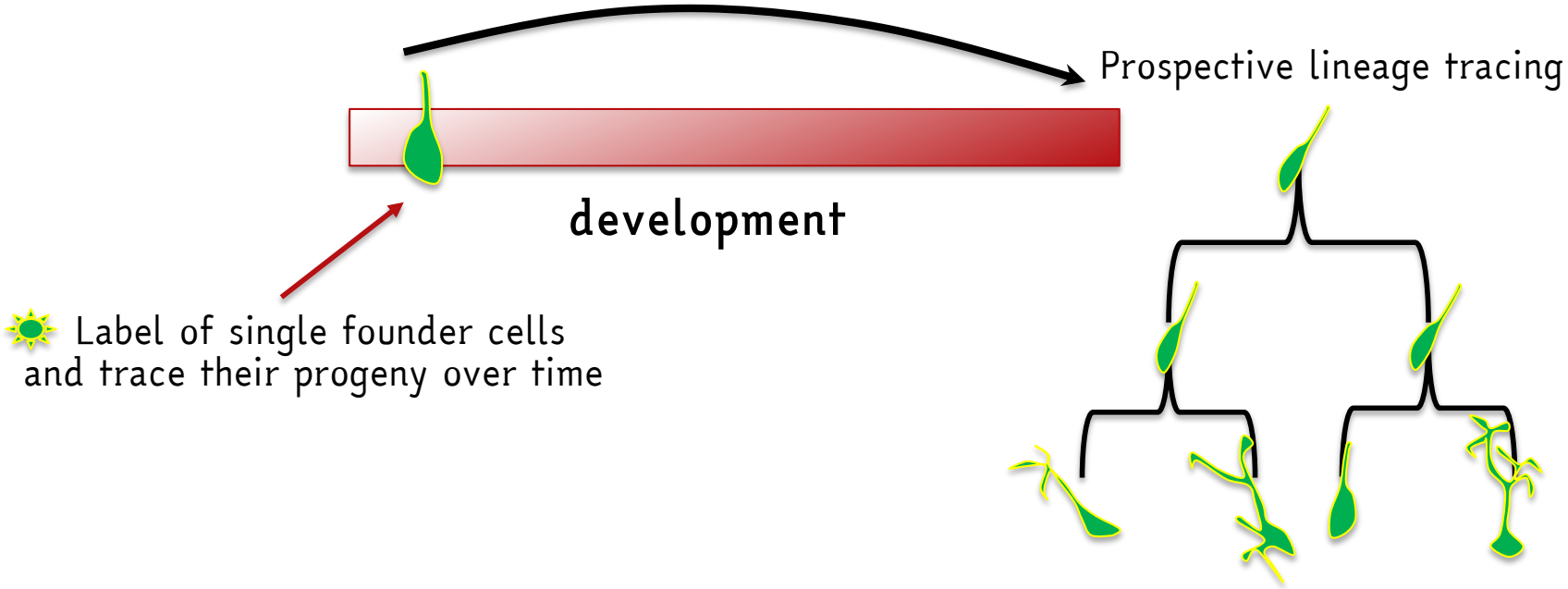
- Provides **spatial and/or temporal resolu**tion to investigate the lineage progression and fate specification of stem cells

Lineage tracing by clonal analysis

- Consists in **quantitative analyses** of clones originated from a single stem/progenitor cell

- Allows for **specific labeling** of single stem/progenitors

- Provides **spatial and/or temporal resolu**tion to investigate the lineage progression and fate specification of stem cells



Genetic tools for prospective lineage tracing :

✓ **Cre-Reporter lines**

✓ **Mosaic expression of multiple genes:**

- Brainbow technology
- Confetti mice
- Star-Track plasmids

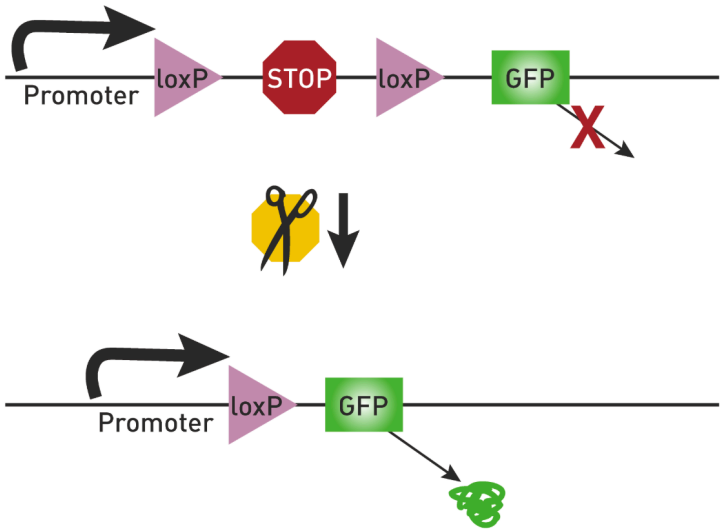
✓ **Retroviral vector-mediated gene transfer**

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

Genetic tools for prospective lineage tracing :

✓ Cre-Reporter lines

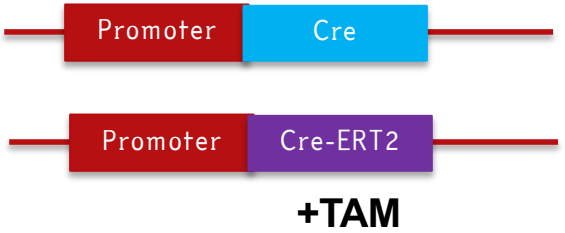
Reporter-lines



X

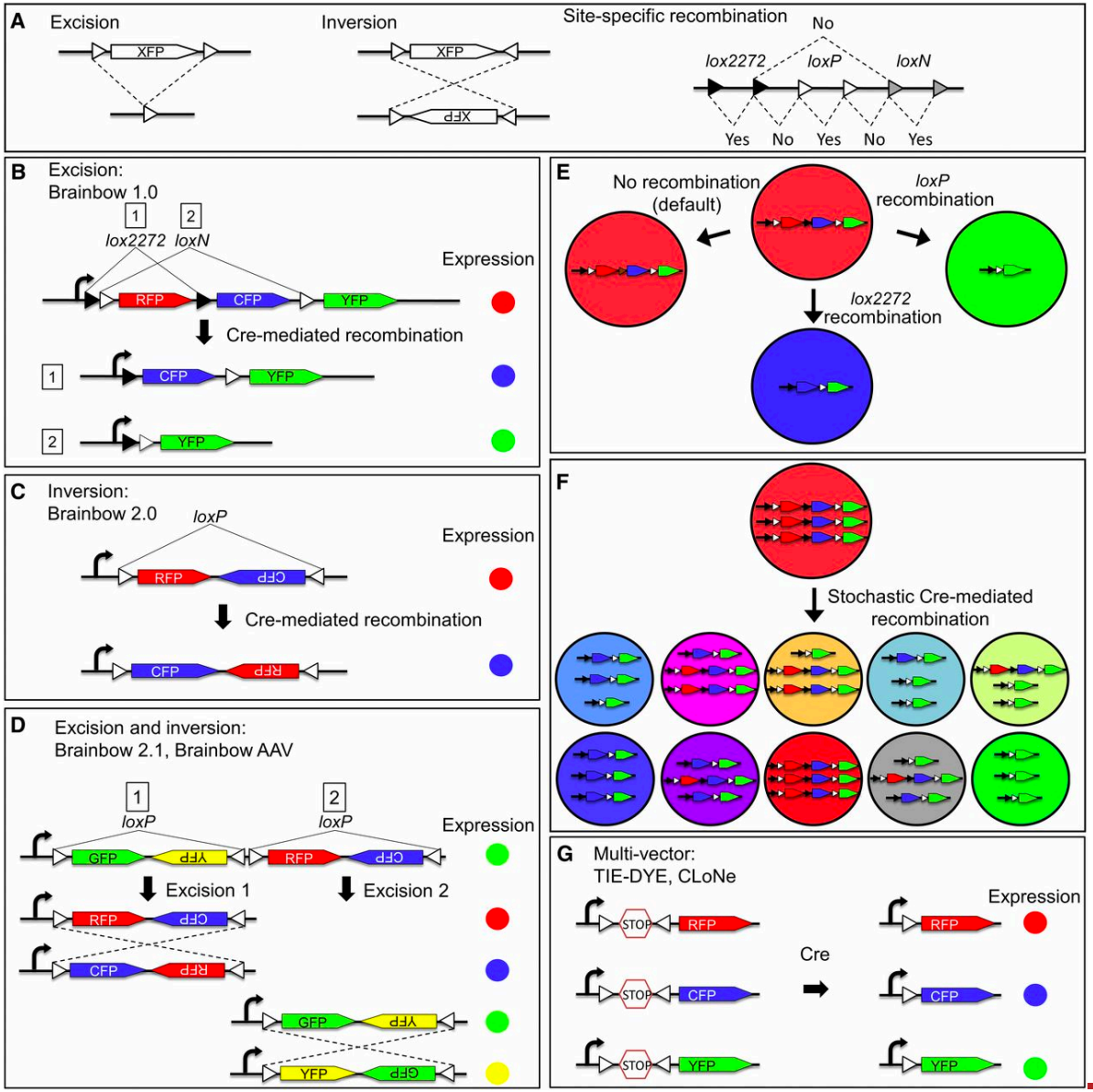
Cre-lines

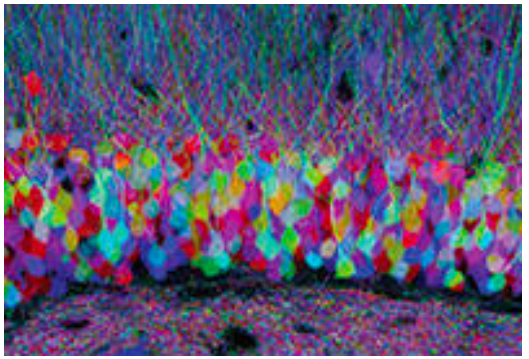
Conditional - Inducible



✓ Mosaic expression of multiple genes: based on Cre/lox recombination system

Brainbow technology





Stochastically express two to four XFPs in a cellular population from a single promoter

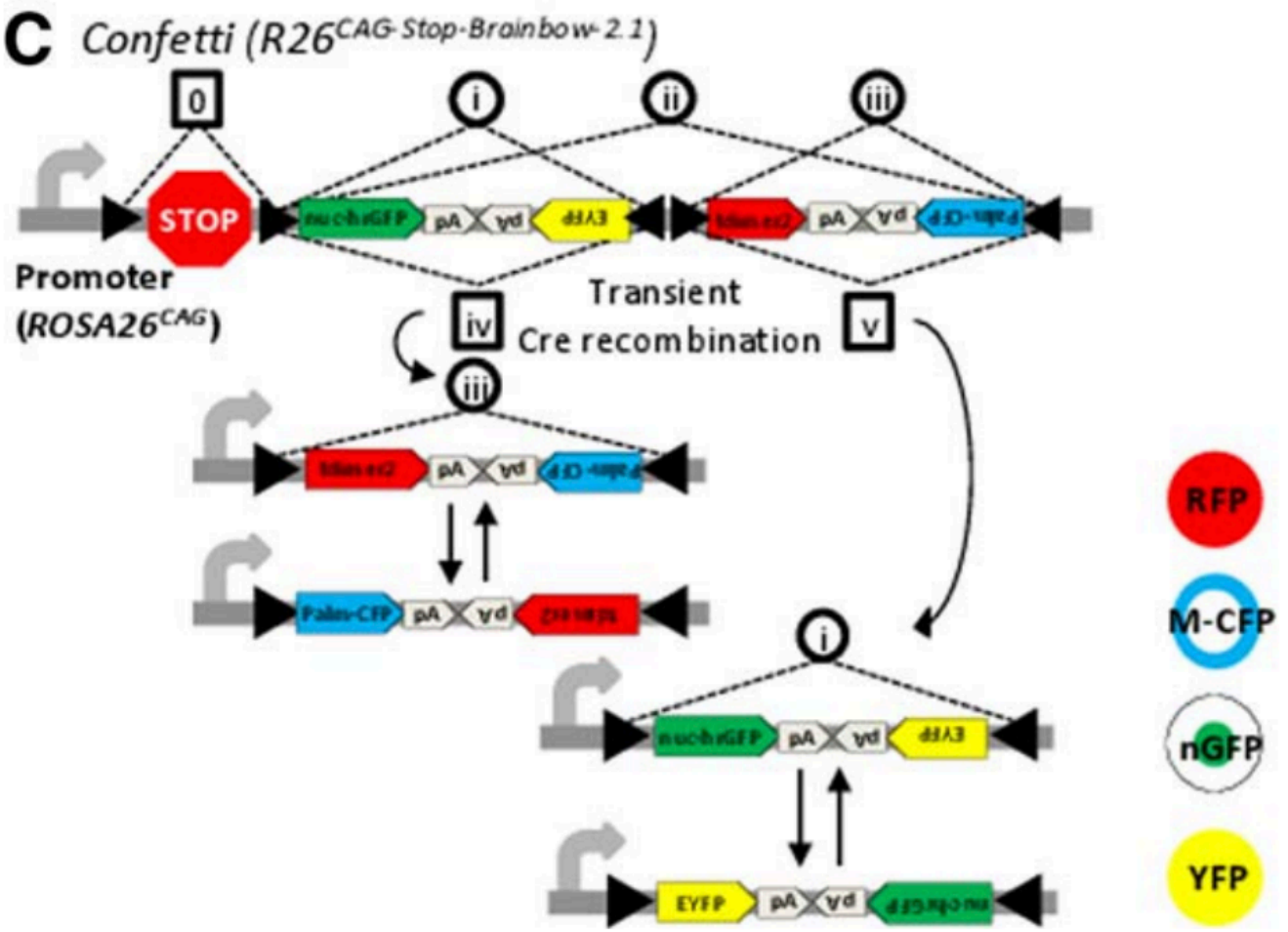
When multiple copies of Brainbow are present in a cell, each copy recombines independently.

3 copies of Brainbow can generate 10 distinct colors and more copies will generate even greater color diversity.

start@unito

✓ Mosaic expression of multiple genes:

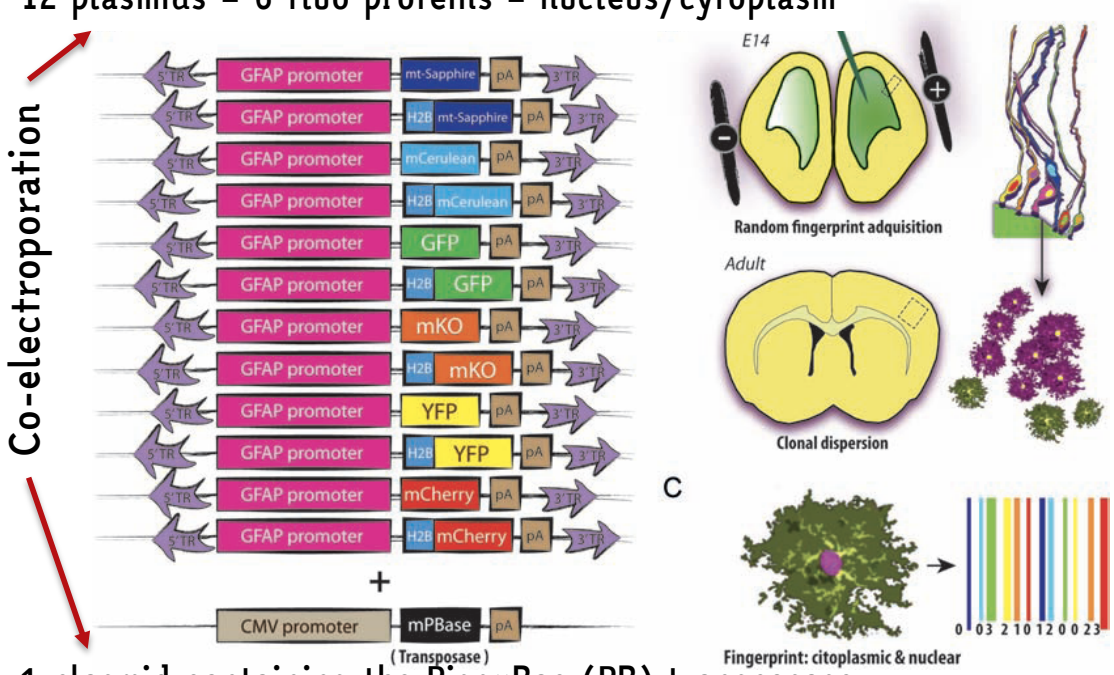
R26R Confetti Mice (Brainbow 2.1)



✓ Mosaic expression of multiple genes:

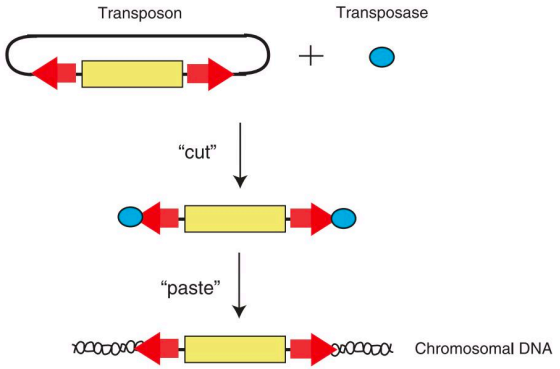
Star Track method – a genetic tracing strategy for long term lineage of individual glial progenitors in vivo -

12 plasmids – 6 fluo proteins – nucleus/cytoplasm



1 plasmid containing the PiggyBac (PB) transposase

Based on the **combinatorial expression** of different fluorescent proteins driven by the GFAP promoter following *in utero* electroporation (the construct corresponding to GFAP-XFP-pA was inserted between the inverted terminal repeats of the PiggyBac transposon)

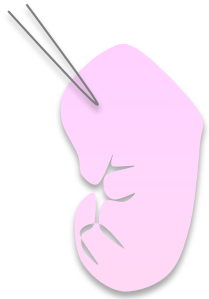


→ Following PBase-mediated transposition, fluorophores are randomly integrated into the cell genome

Genetic tools for prospective lineage tracing :

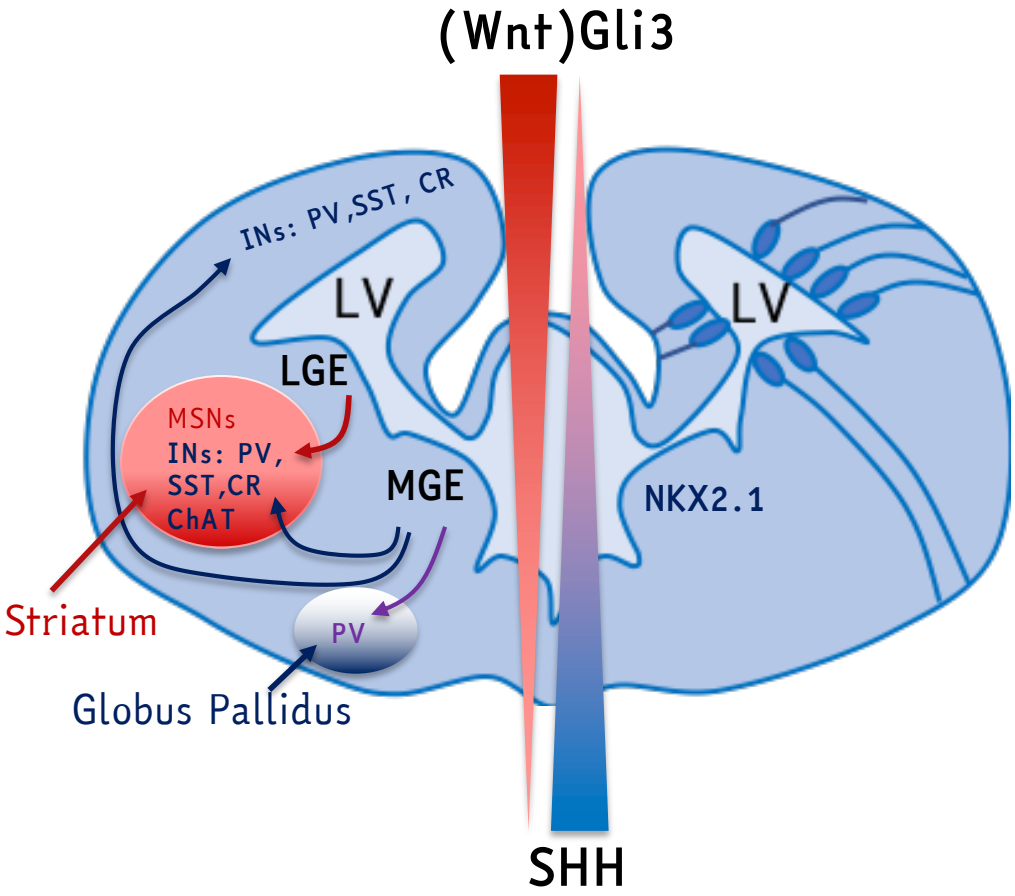
- ✓ **Retroviral vector-mediated gene transfer**
- RV carrying reporter genes (i.e. GFP)
- Barcoded Retroviral libraries (i.e. QmGFP-OL)

Retrovirus iv injection

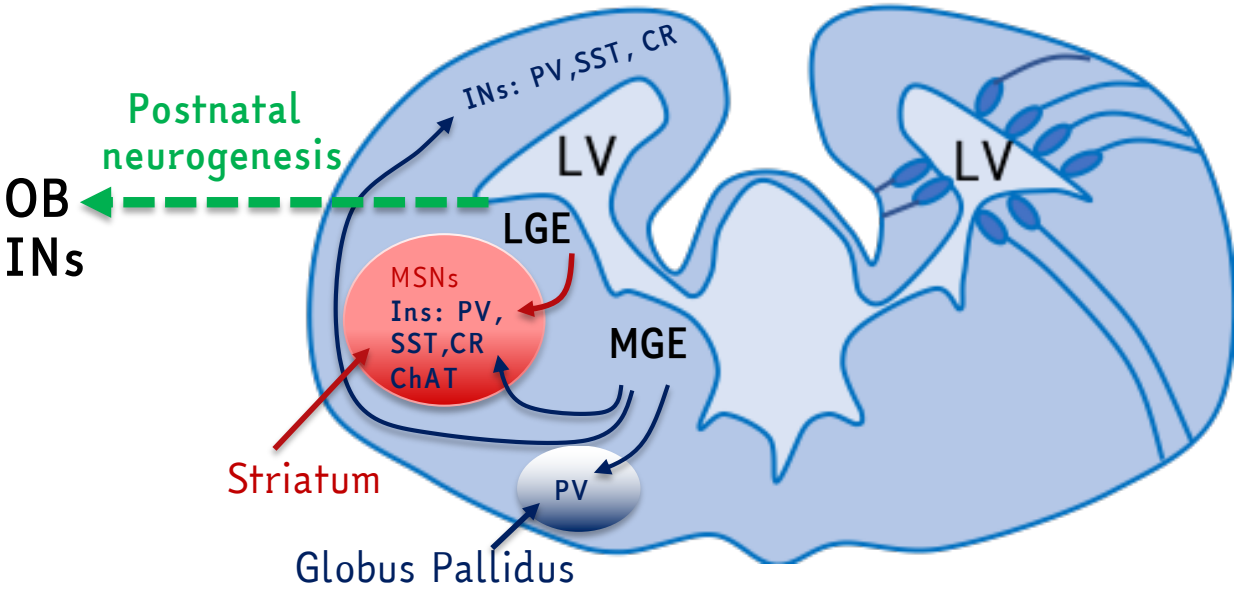


- allows viruses to introduce recombinant DNA into the genome of a host cell (proliferating)
- the integrated exogenous DNA is then inherited by all the descendants of the infected cell

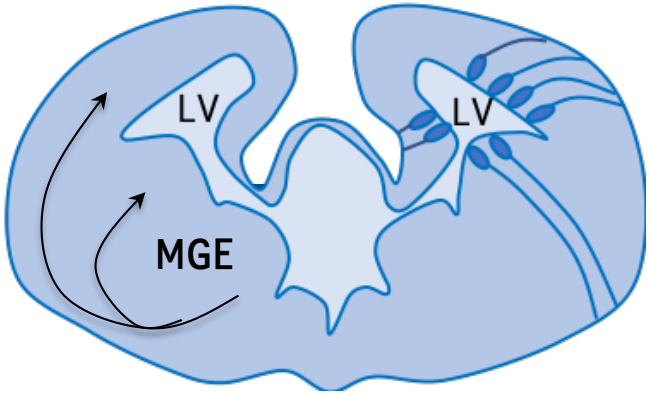
Multiple progenitor domains in the developing telencephalon



Multiple progenitor domains in the developing telencephalon



Lineage clonal analysis using Retroviral vector-mediated gene transfer to study MGE-derived inhibitory interneurons

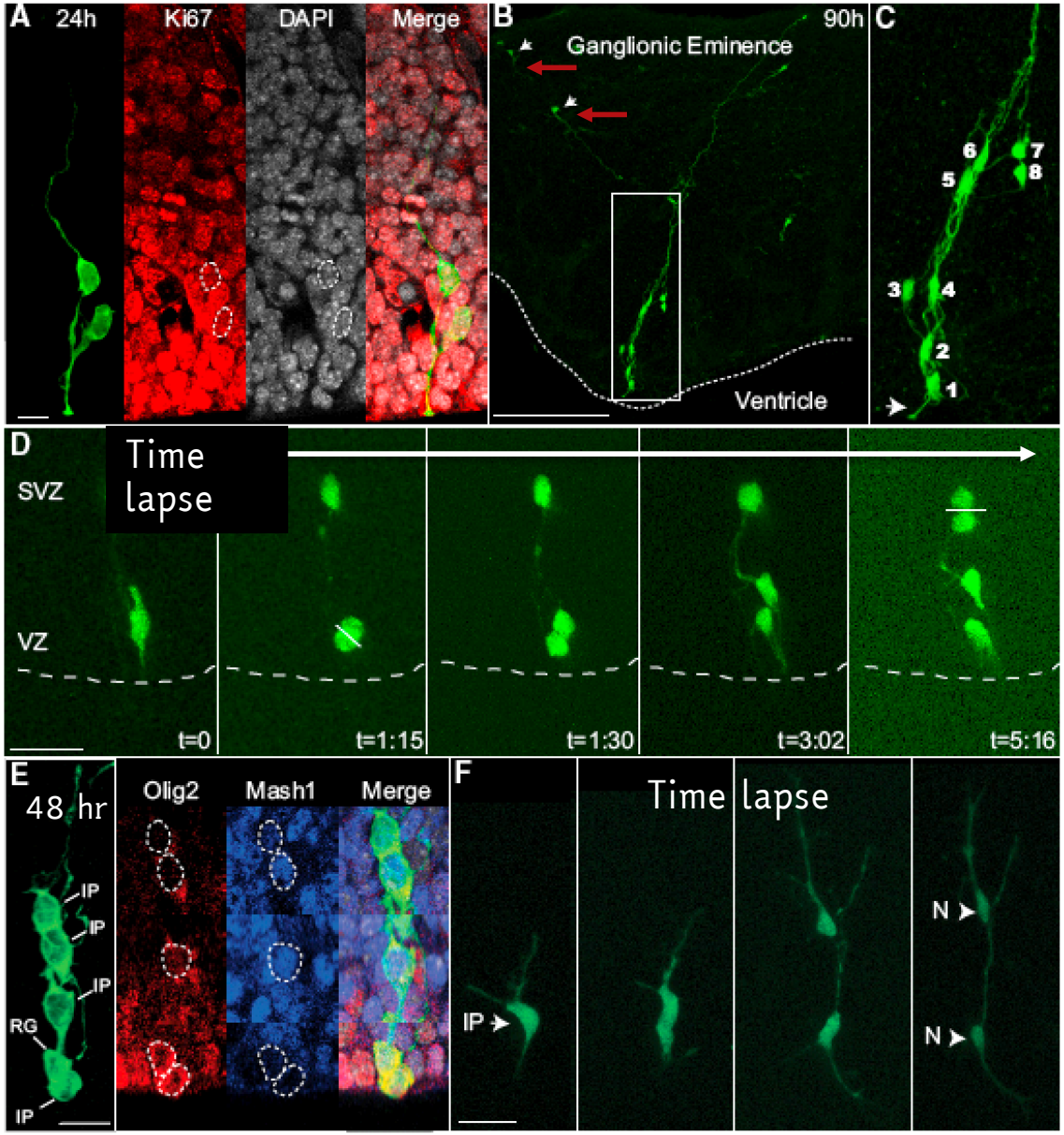


Neuron
Article

Wide Dispersion and Diversity of Clonally Related Inhibitory Interneurons

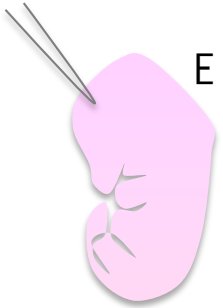
Corey C. Harwell,[1,*] Luis C. Fuentealba,[2,5] Adrian Gonzalez-Cerrillo,[1] Phillip R.L. Parker,[4] Caitlyn C. Gertz,[3,5] Emanuele Mazzola,[6] Miguel Turrero Garcia,[1] Arturo Alvarez-Buylla,[2,5] Constance L. Cepko,[7] and Arnold R. Kriegstein[3,5]
[1]Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA
[2]Department of Neurological Surgery
[3]Department of Neurology
University of California, San Francisco, San Francisco, CA 94143, USA
[4]Gladstone Institute for Neurological Disease, San Francisco, CA 94158, USA
[5]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, San Francisco, CA 94143, USA
[6]Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, USA
[7]Departments of Genetics and Ophthalmology and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: corey_harwell@hms.harvard.edu
http://dx.doi.org/10.1016/j.neuron.2015.07.030

Harwell et al., Neuron 2015



Intraventricular retroviral injection

RV-GFP



E 12.5

- RGCs
- IPC
- Newborn neurons

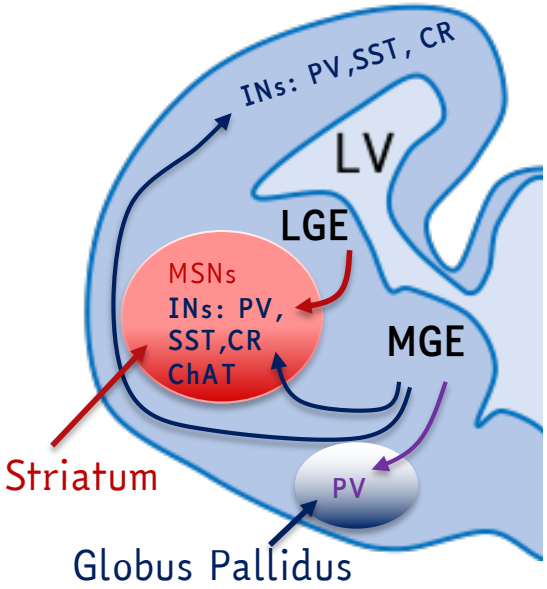
Ki67 = cell proliferation marker

Organotypic slices
24 h after injection

bHLH TFs
Olig2
Mash1

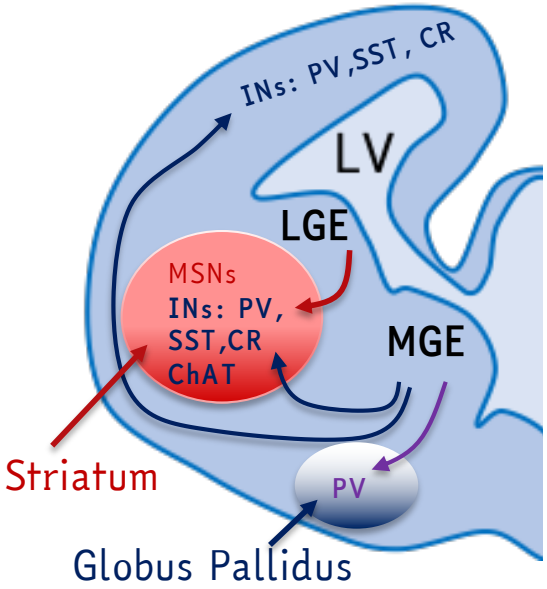
→ Ventral Telencephalic Progenitor Markers

IPC divisions contribute to the expansion of neuronal lineages in the MGE



RV-GFP intraventricular injection allows clonal lineage analysis BUT only if the progeny remains spatially confined

Need for an alternative method to follow the distribution of cells with broad migratory capacity in relation to their clonal siblings

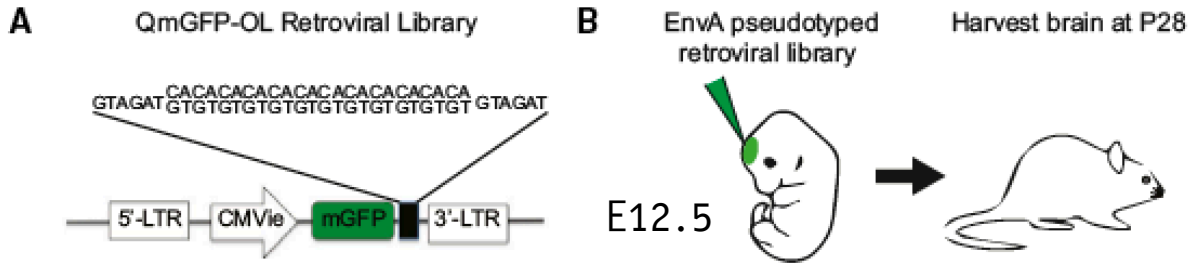


RV-GFP intraventricular injection allows clonal lineage analysis BUT only if the progeny remains spatially confined

Need for an alternative method to follow the distribution of cells with broad migratory capacity in relation to their clonal siblings

Barcoded Retroviral libraries

EnvA-pseudotyped retrovirus library carrying oligonucleotide sequence tags or barcodes



Schematic of the QmGFP-OL murine retroviral library.

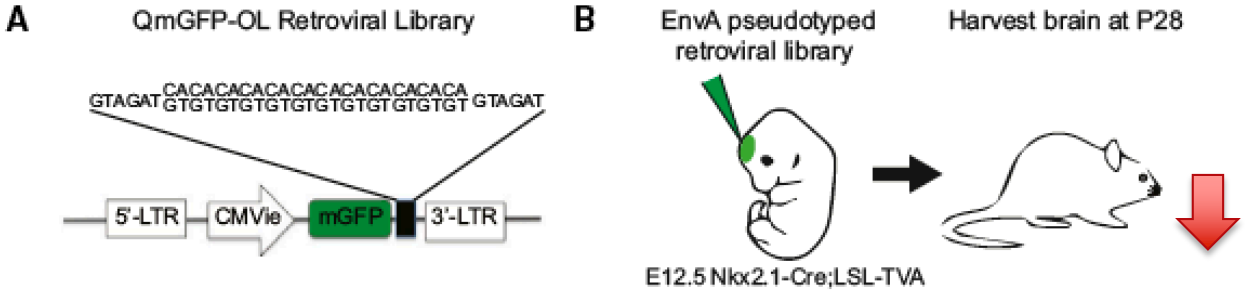
→ Each retrovirus expresses membrane GFP and contains a 24-bp barcode sequence

10^5 unique 24-bp barcode sequences

Cells were labeled from each injection (n = 8 hemispheres, 4 brains) - between 14 and 234

The probability of neurons containing the same tag arising from two independent progenitor infections is low

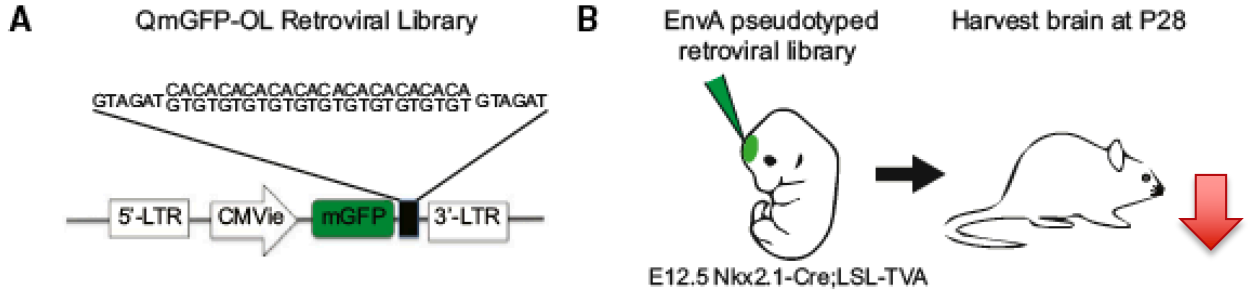
→ Multiple cells sharing the same barcode are presumed to be clonally related siblings



Brain sectioning

Immunofluorescence for INs subtype markers PV and SOM

Spatial mapping of GFP/PV+ and GFP/SOM + cells



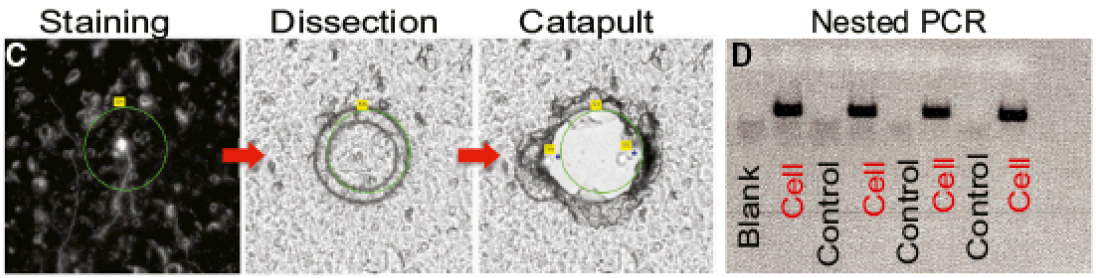
Brain sectioning

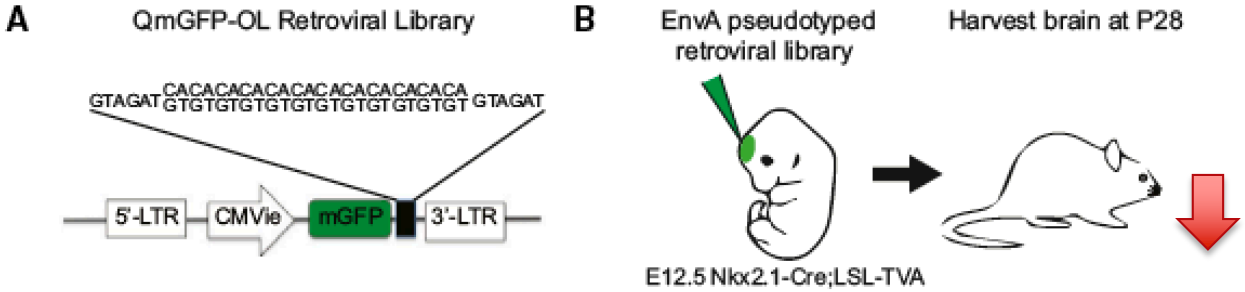
Immunofluorescence for INs subtype markers PV and SOM

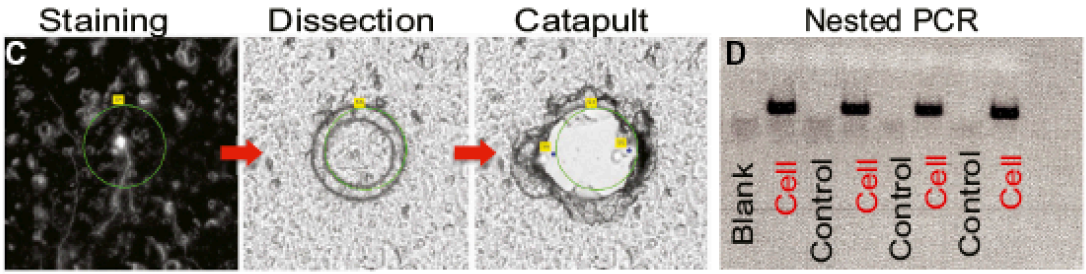
Spatial mapping of GFP/PV+ and GFP/SOM + cells

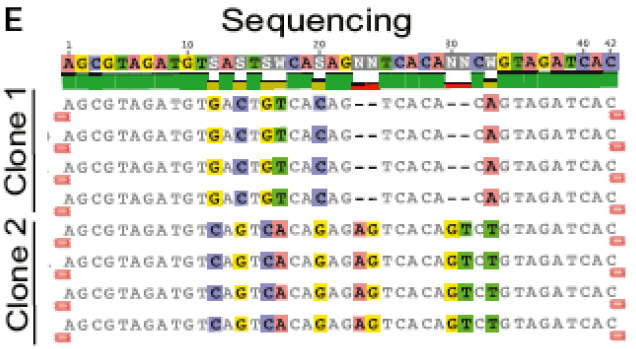
Laser capture microdissection of labelled cells

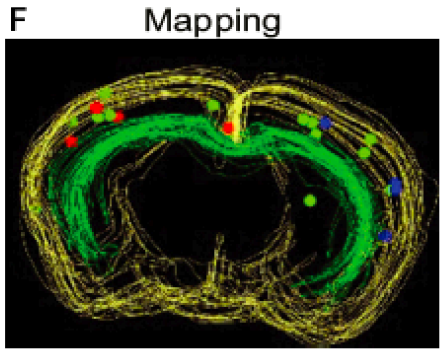
Viral tag amplification by PCR











Brain sectioning

Immunofluorescence for INs subtype markers PV and SOM

Spatial mapping of GFP/PV+ and GFP/SOM + cells

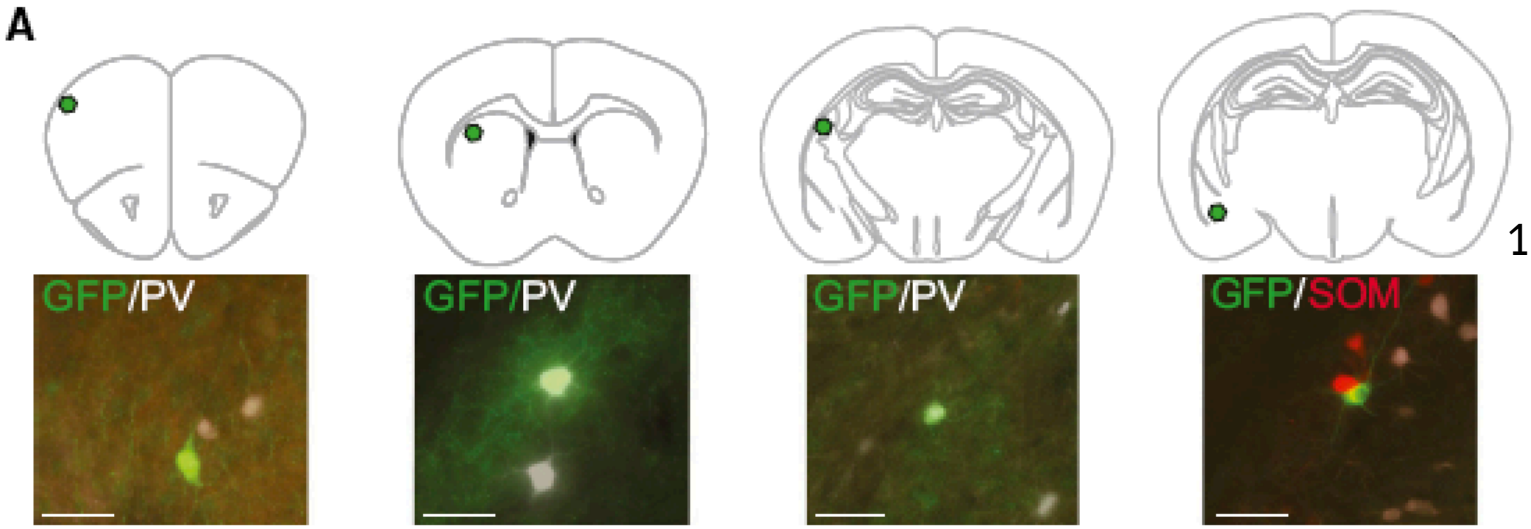
Laser capture microdissection of labelled cells

Viral tag amplification by PCR

Sequencing

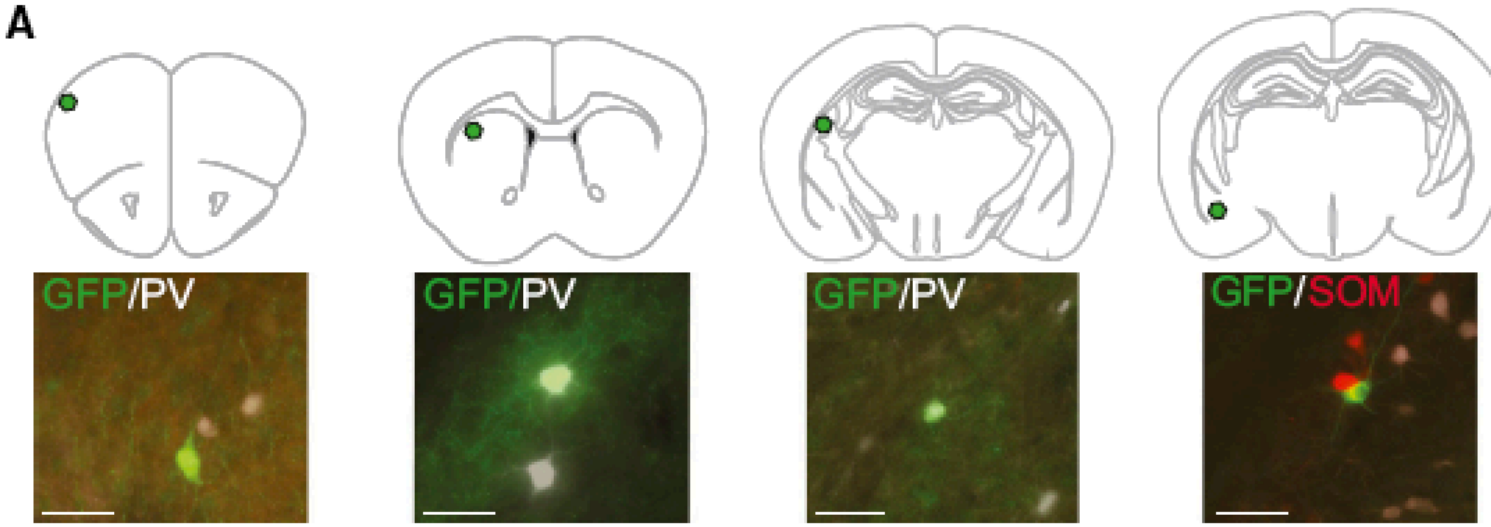
3D map of the GFP+ cells

Interneuron Clones Consist of Widely Dispersed Cells of Diverse Subtypes

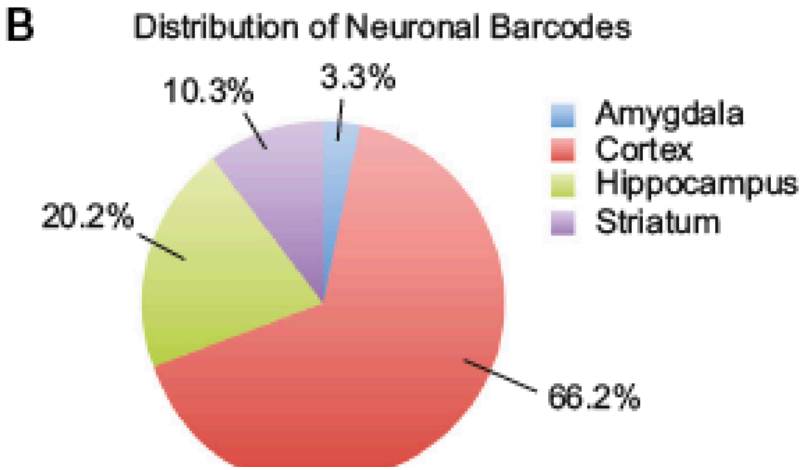


1 clone = 4 cells

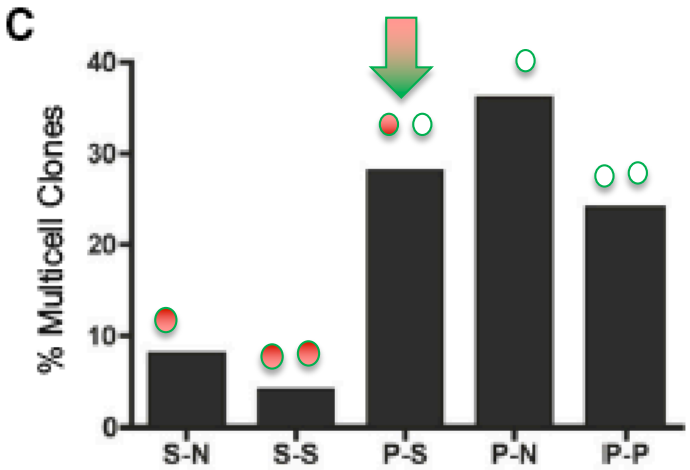
Interneuron Clones Consist of Widely Dispersed Cells of Diverse Subtypes



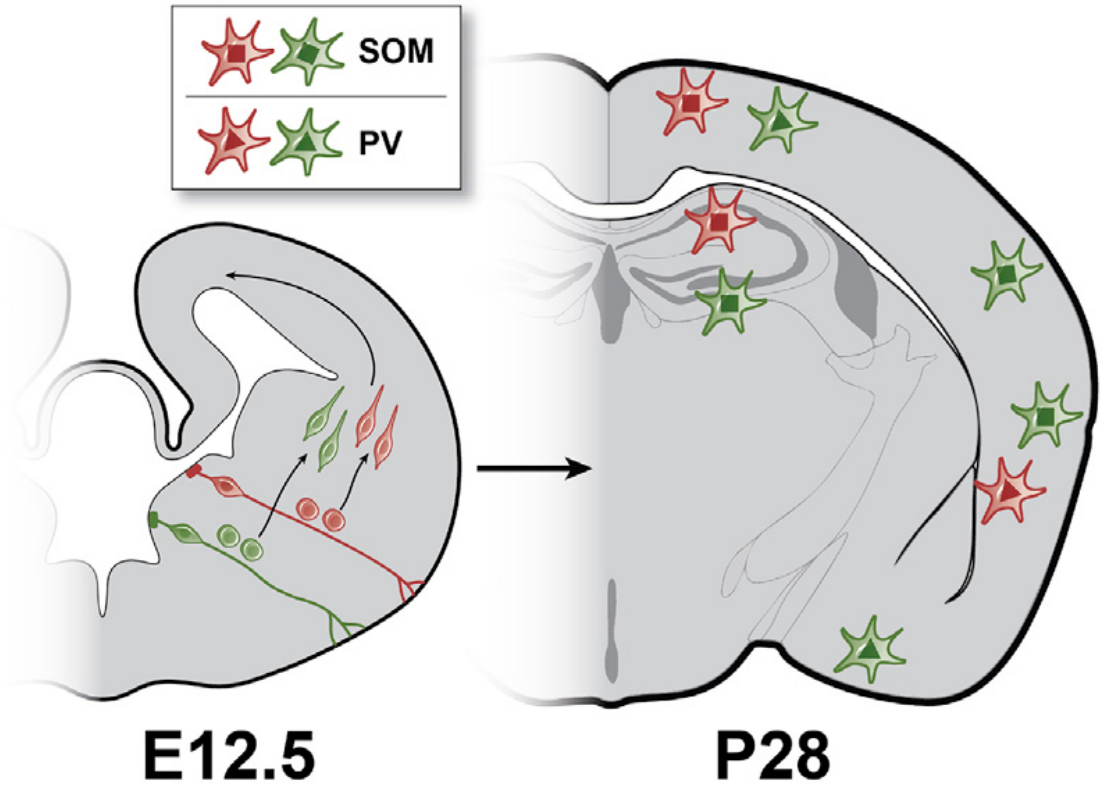
1 clone = 4 cells



Pie chart of forebrain regions that contain neurons that returned a barcode (N=302)



Wide Dispersion and Diversity of Clonally Related Interneurons



MGE interneurons derived from a common progenitor are widely dispersed across different regions of the brain

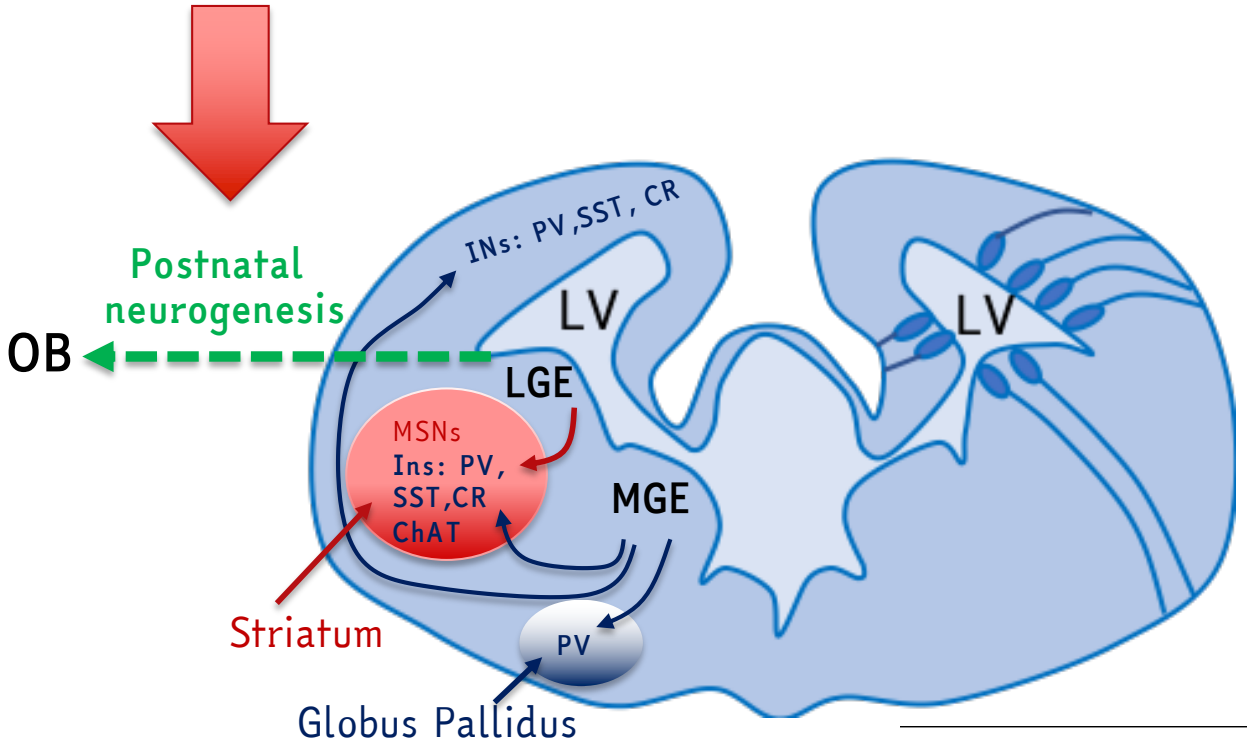
→the majority of **clustered cells observed with sparse viral labeling are likely not clonally related**

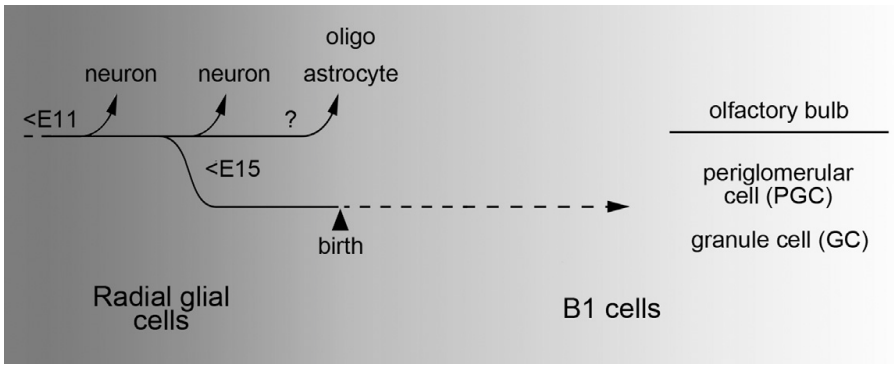
Barcoded Retroviral libraries (i.e. QmGFP-OL)

Advantages: allow determining lineage relationships in populations of cells regardless of their pattern of migration

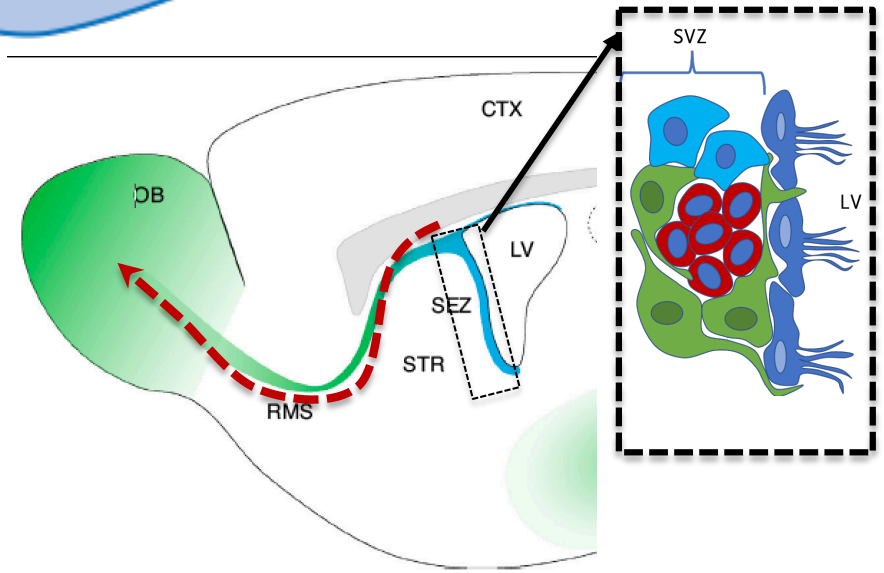
→tags recovered from only 43% of GFP-positive cells

Limits: gene silencing →can account for some reduction in clone size

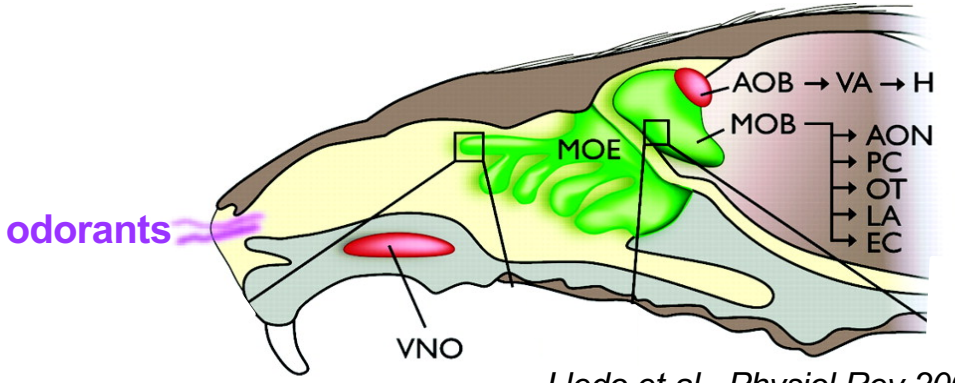




Fuentealba et al., 2015



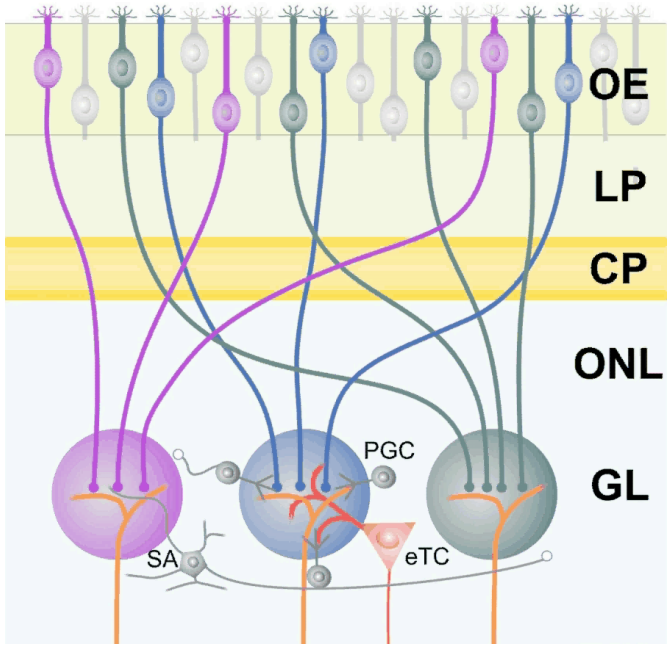
The olfactory system



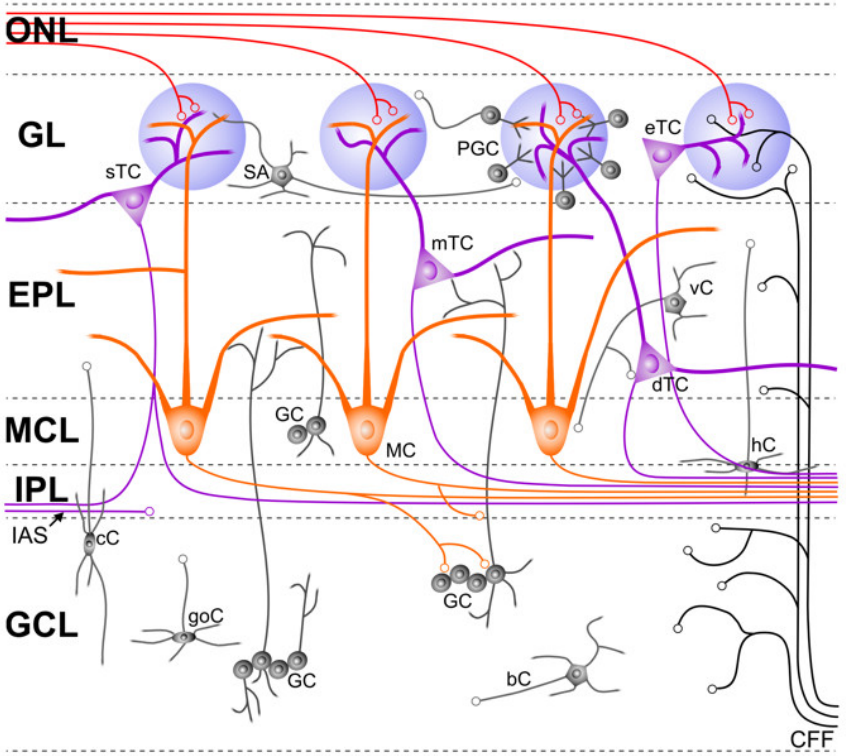
Lledo et al., Physiol Rev 2004

MOE

MOB

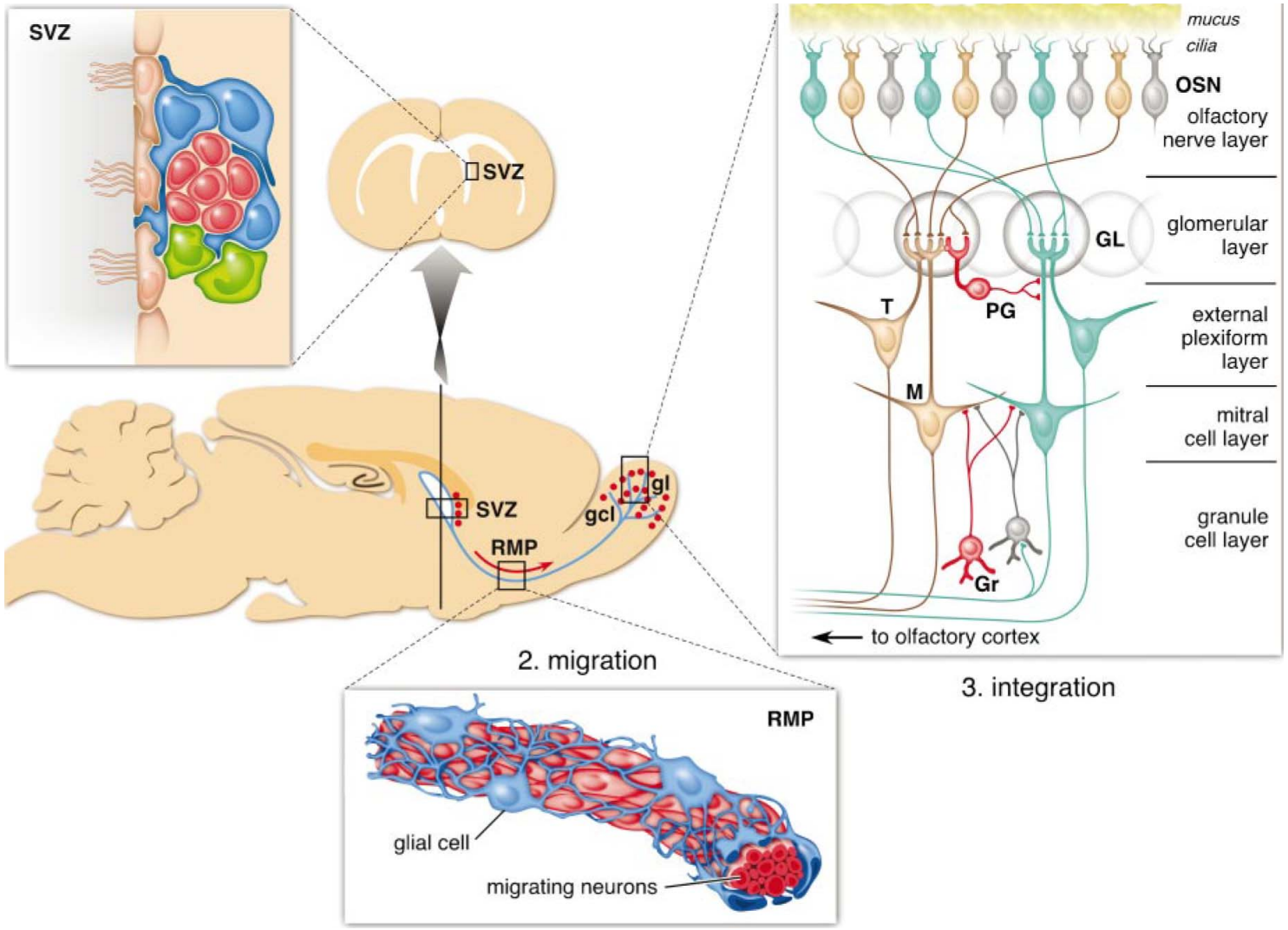


courtesy of A. Puche



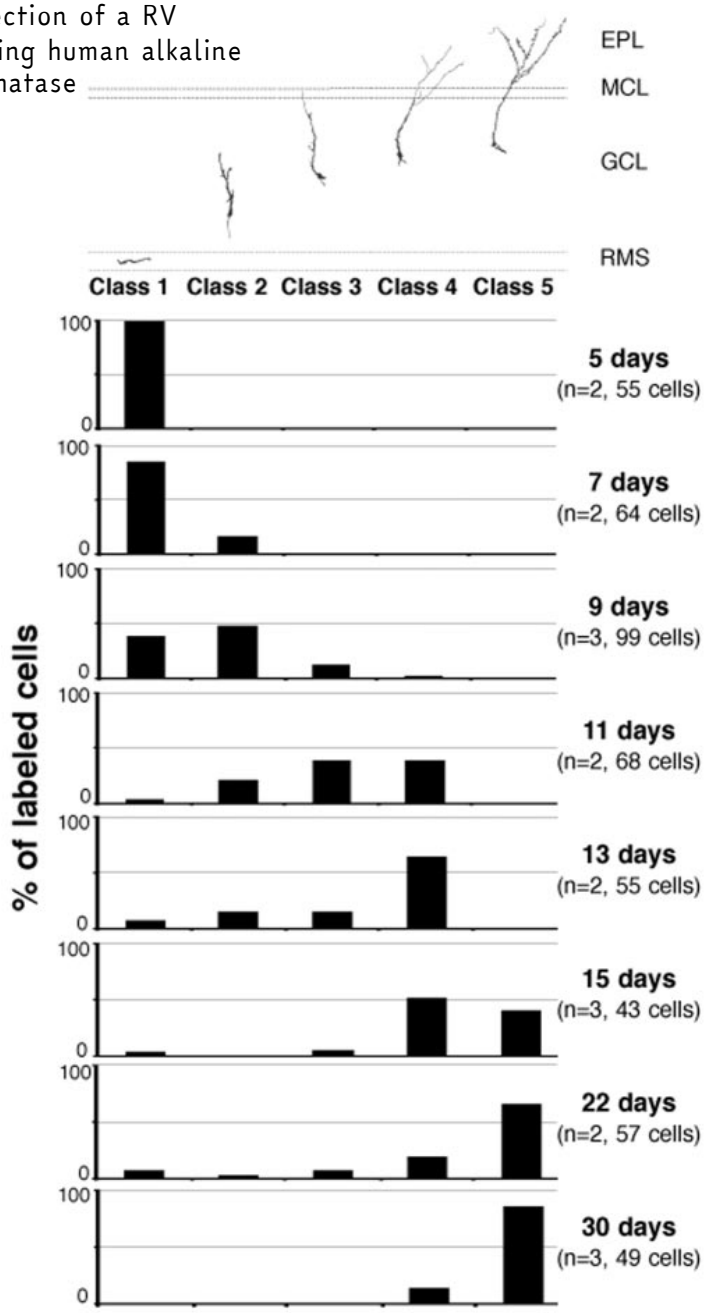
start@unito

Adult neurogenesis: a multi step process



Maturation & selection of adult born OB interneurons

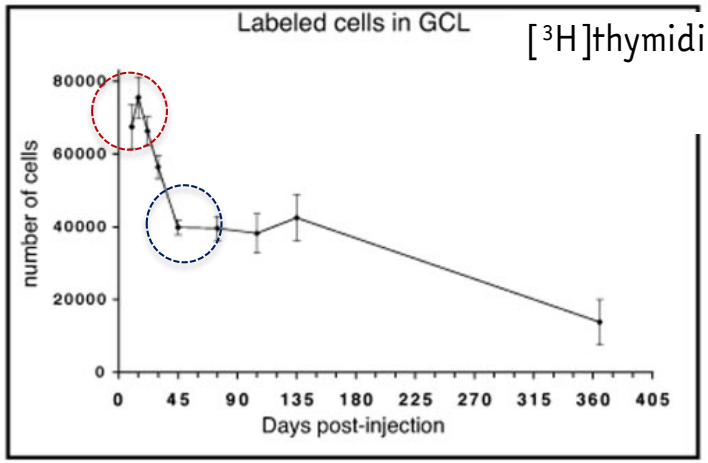
iv injection of a RV encoding human alkaline phosphatase

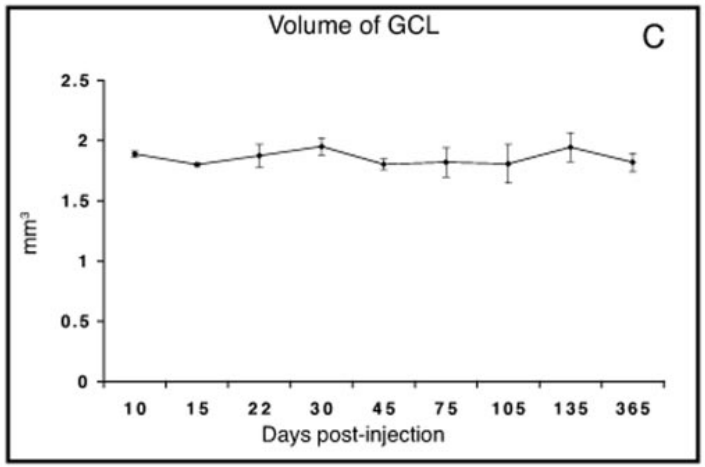


Petreanu et al., J Neurosci 2002

quantitative estimation of the absolute number of new neurons recruited into the bulb

[^{3}H]thymidine





Embryonic Origin of Postnatal Neural Stem Cells

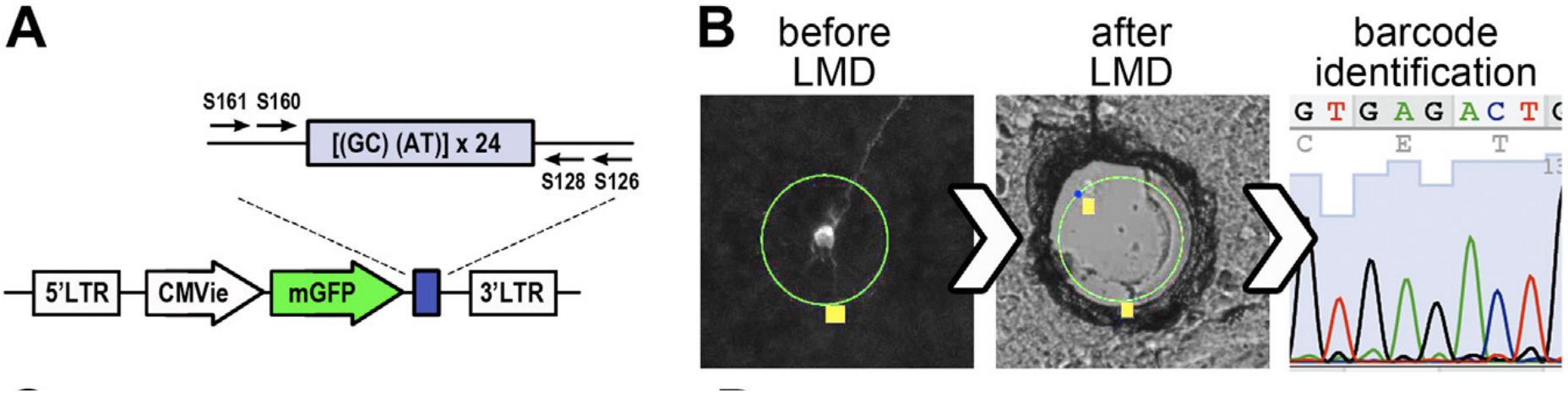
Luis C. Fuentealba,[1] Santiago B. Rompani,[2] Jose I. Parraguez,[1] Kirsten Obernier,[1] Ricardo Romero,[1] Constance L. Cepko,[2] and Arturo Alvarez-Buylla[1,*]

[1]Department of Neurological Surgery and the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA 94143, USA

[2]Departments of Genetics and Ophthalmology and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA

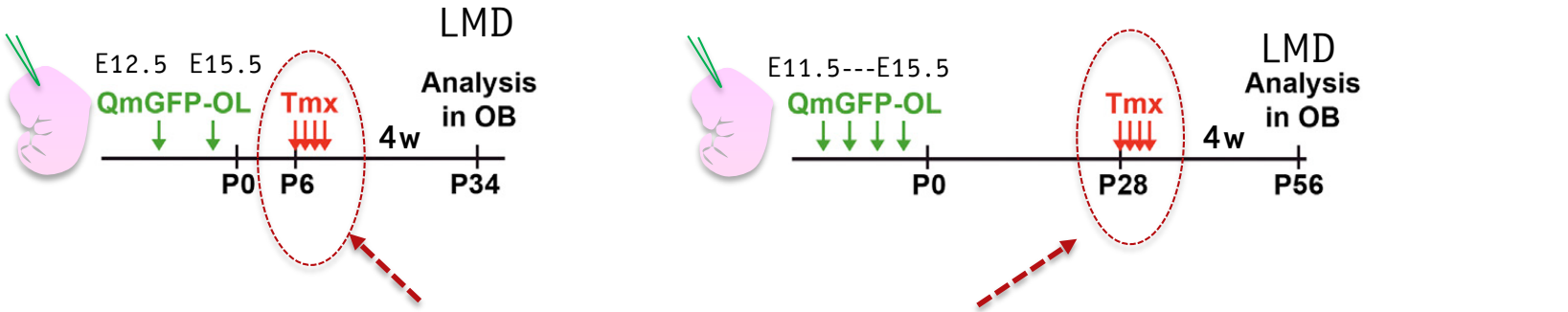
*Correspondence: abuylla@stemcell.ucsf.edu

http://dx.doi.org/10.1016/j.cell.2015.05.041



Retroviral barcode library:
each retrovirus expresses mGFP
and contains a unique 24 bp sequence

Retroviral barcode library injected intraventricularly at different embryonic stages



Nestin-CreER^{T2}/tdTomato-Reporter Mice

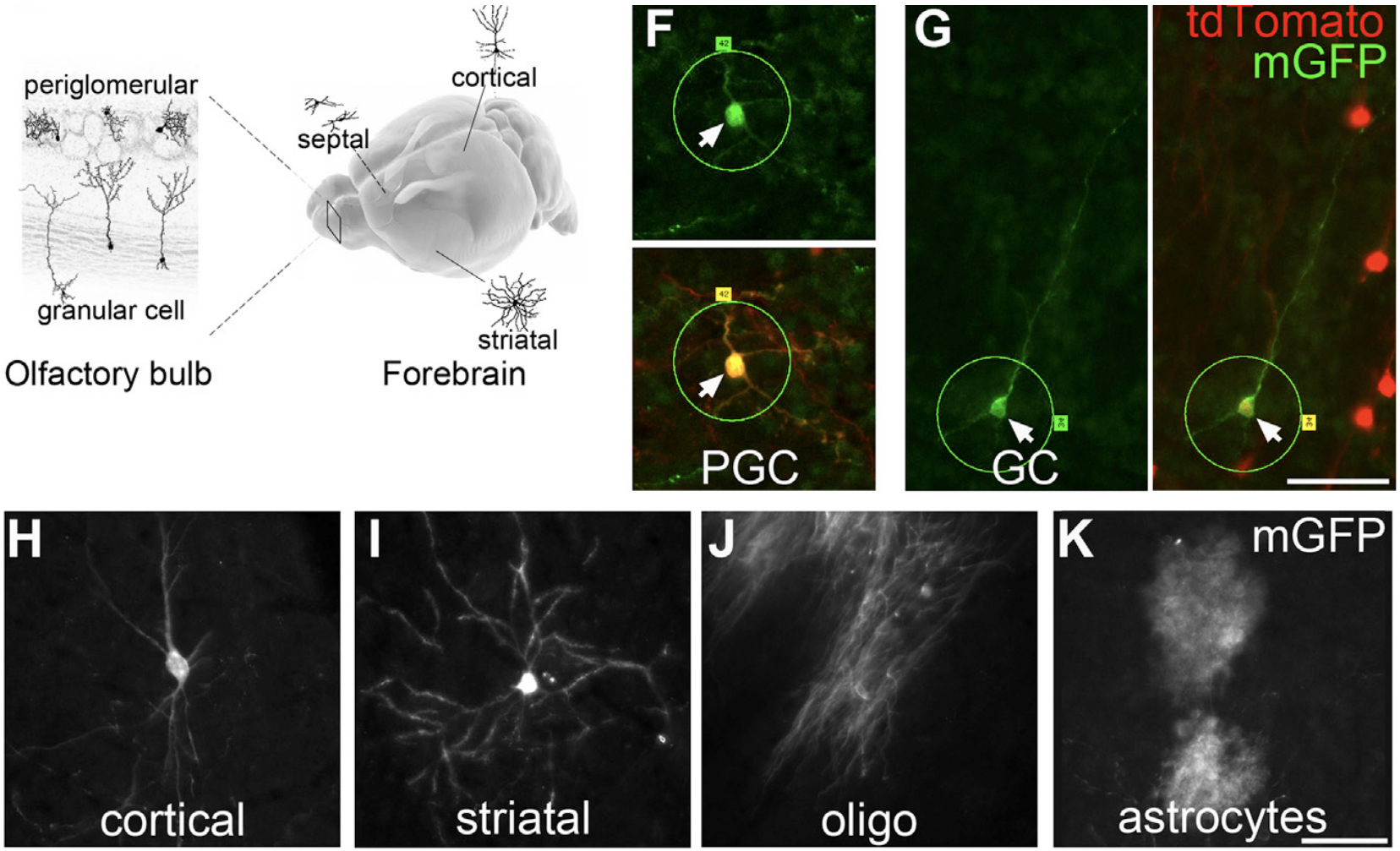
mGFP+
mGFP+TdT+

→ individually labeled cells were identified as neurons, astrocytes, and oligodendrocytes:

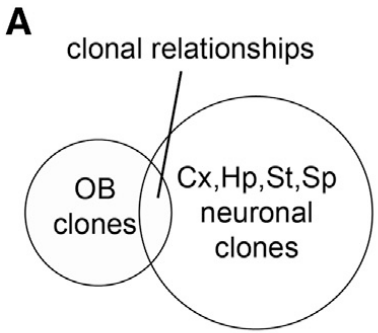
1) based on their **morphology**, revealed by mGFP

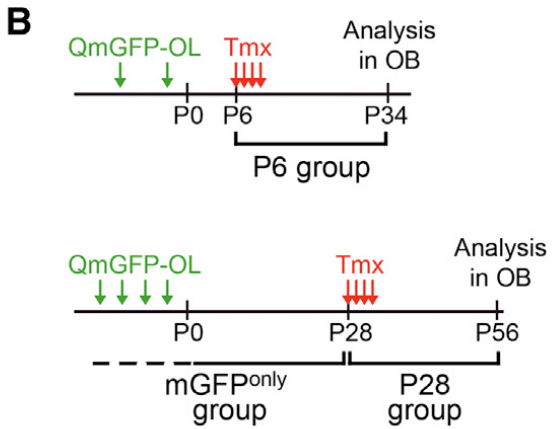
2) by the **expression** of NeuN, GFAP, and Olig2

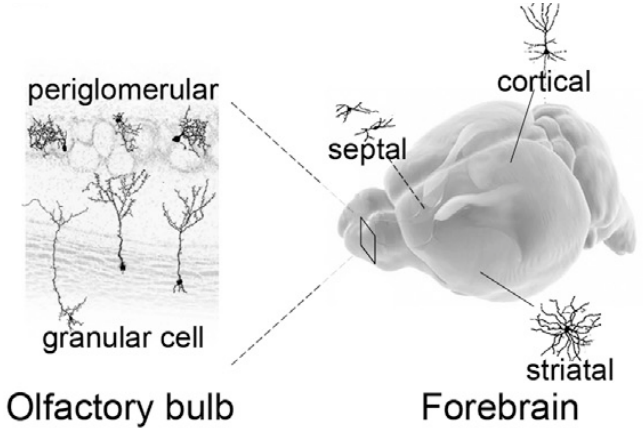
Their locations were mapped, and individual labeled cells were collected using laser capture

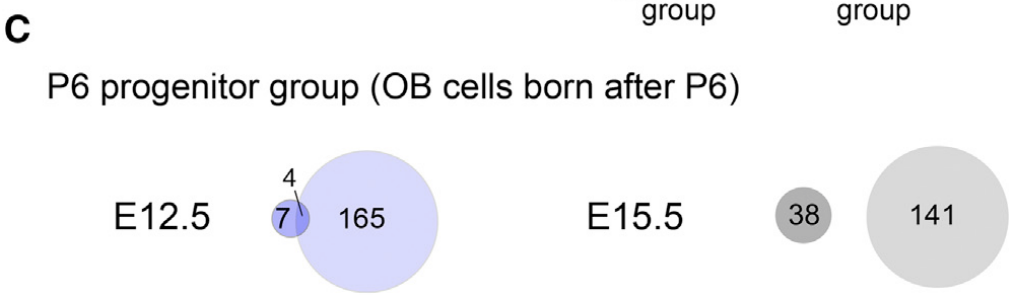

periglomerular
granular cell
Olfactory bulb
septal
cortical
striatal
Forebrain
F
PGC
G
GC
tdTomato
mGFP
H
cortical
I
striatal
J
oligo
K
mGFP
astrocytes

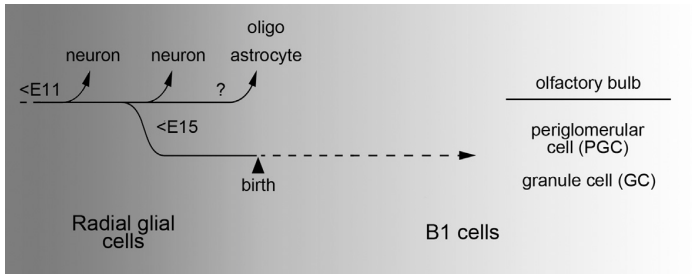
Clonal Relationships between Postnatal OB Interneurons and Cx, Hp, St, and Sp Neurons

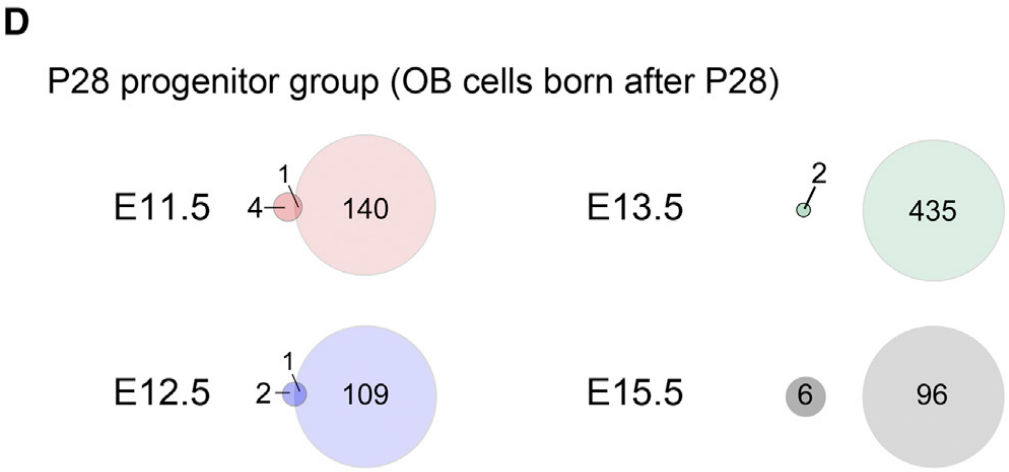


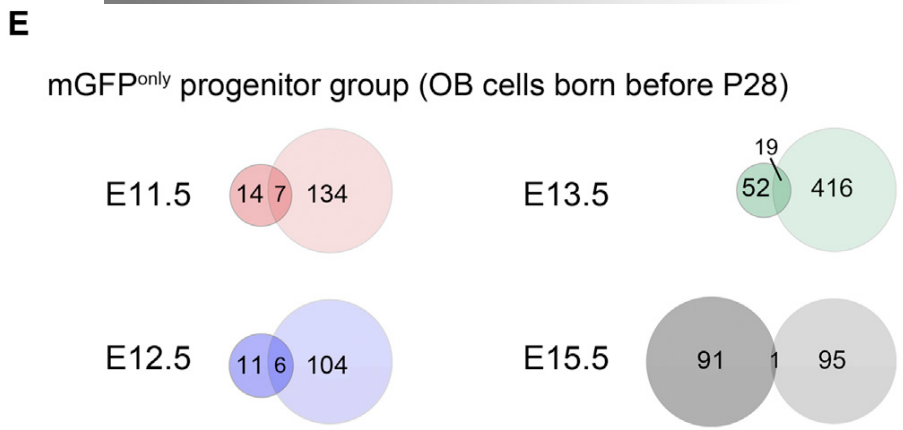




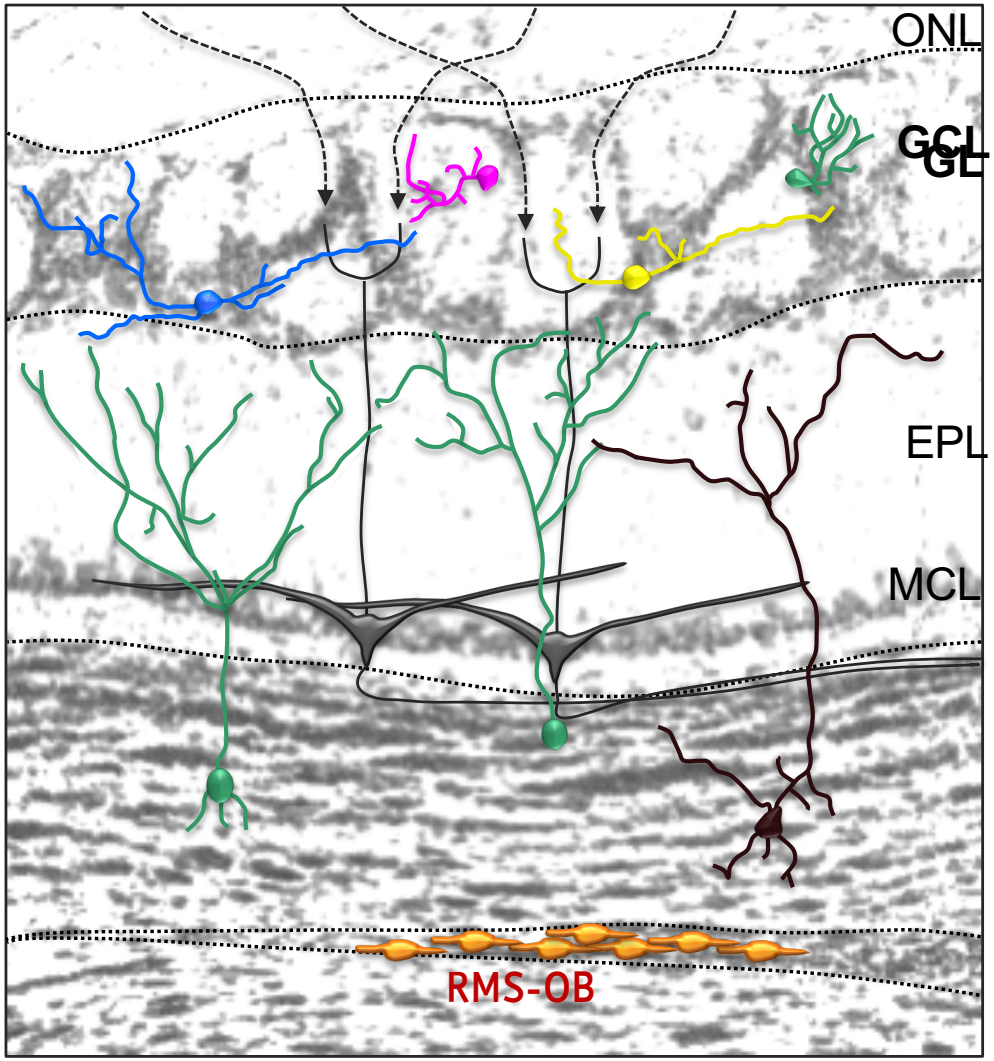






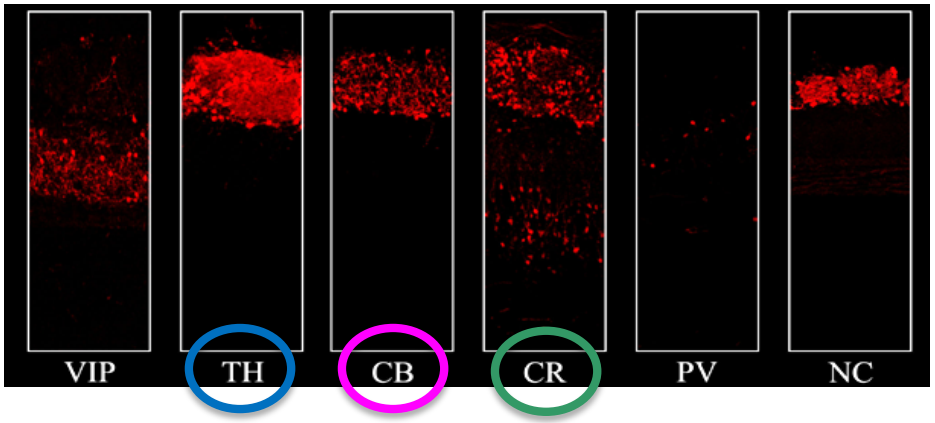


Inhibitory GABA-ergic Interneurons belong to different sub-types



GL, glomerular layer; **GCL**, granule cell layer

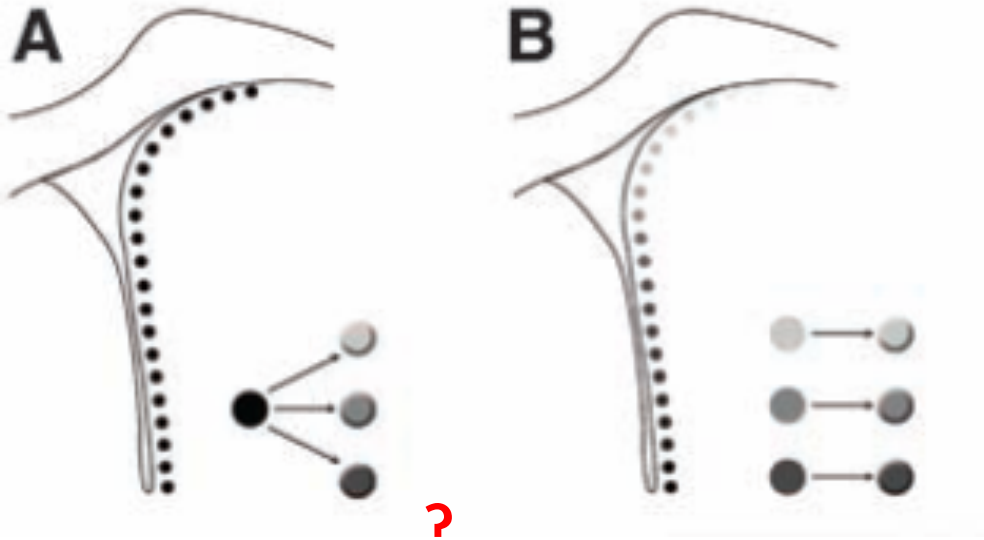
Neurochemical phenotypes
of OB GABAergic interneurons



Science
AAAS

Mosaic Organization of Neural Stem Cells in the Adult Brain
Florian T. Merkle, *et al.*
Science **317**, 381 (2007);
DOI: 10.1126/science.1144914

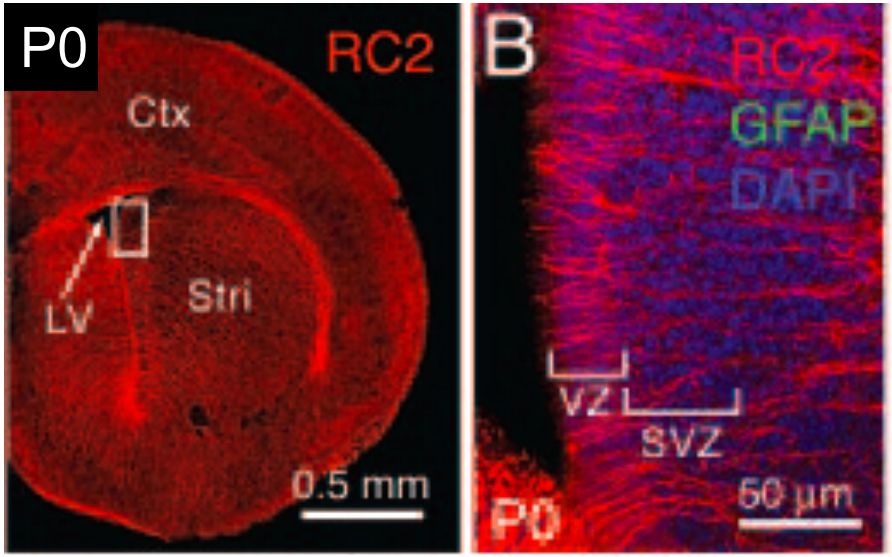
Model of SVZ stem cell potential

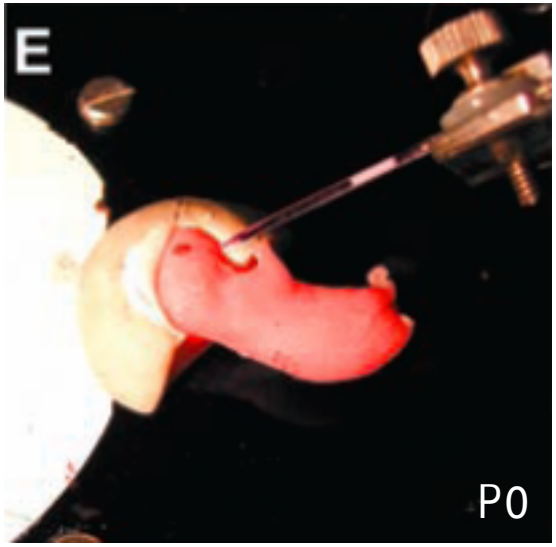


Equivalent stem cells generate multiple neuron types

?
OR

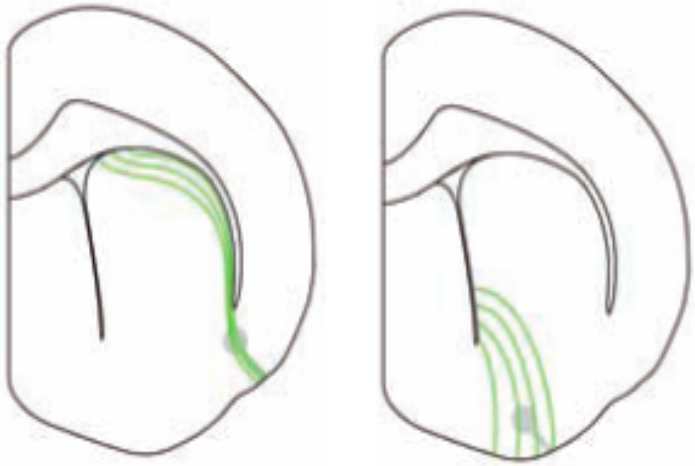
Different stem cells generate specific neuron types





(Merkle et al., 2007)

label radial glia
in a regionally specific manner

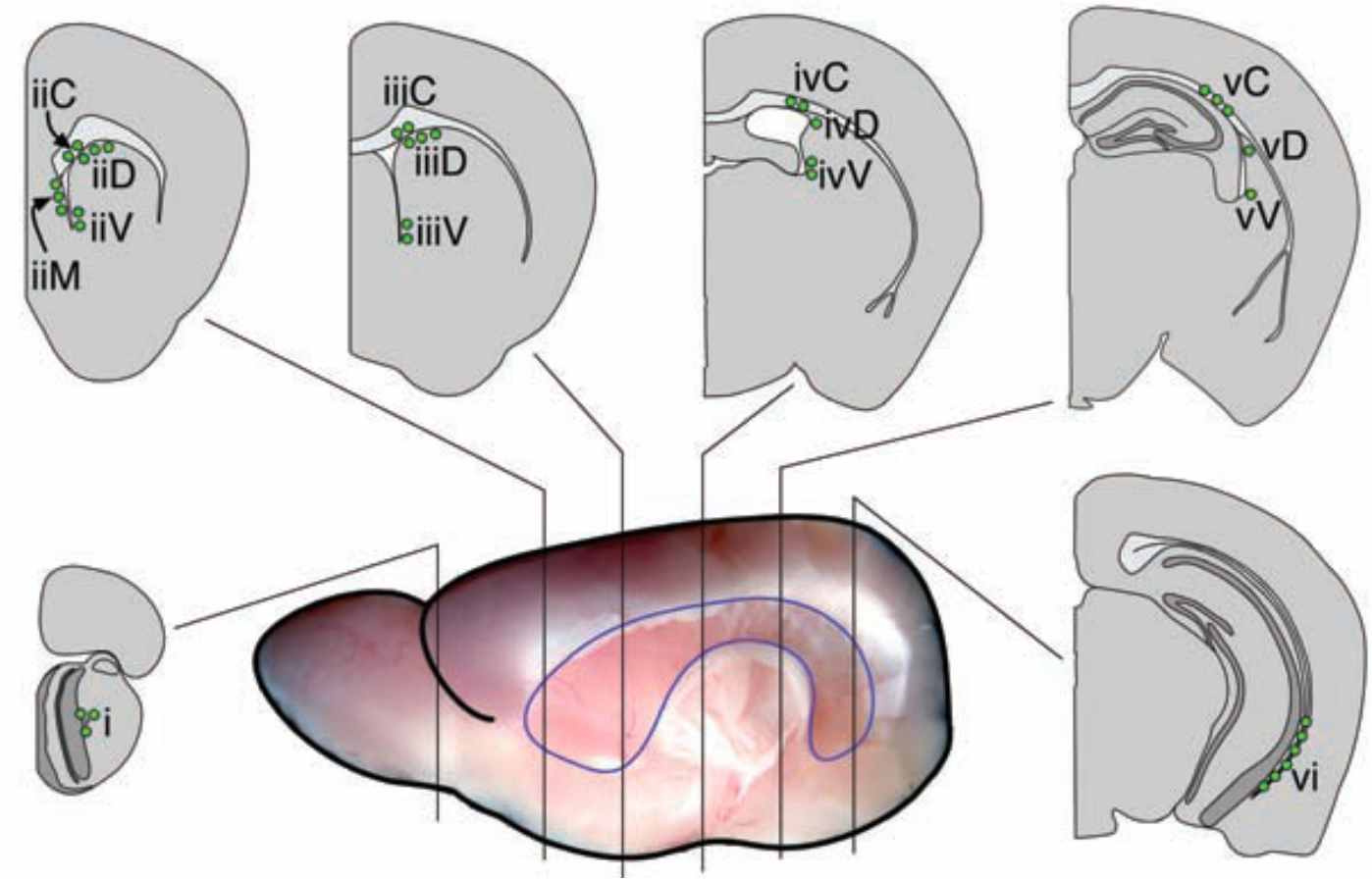


Specifically targeting stem cells and follow their progeny in vivo by Ad-Cre (Adenovirus expressing Cre recombinase) injection into GFP reporter mice.

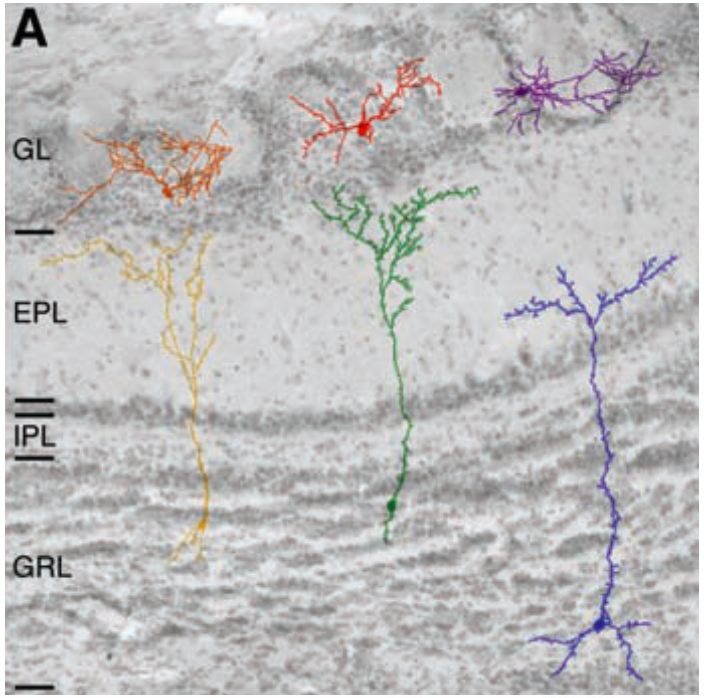
Little diffusion → localized injection (small volumes)

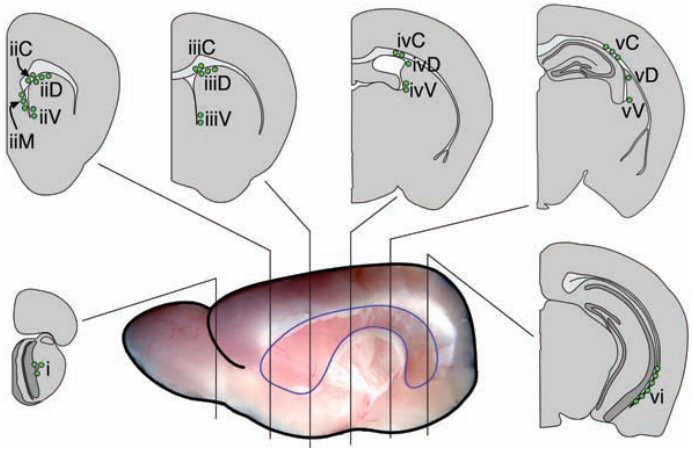
Ad-Cre infected radial glia and their progeny become permanently labeled with GFP

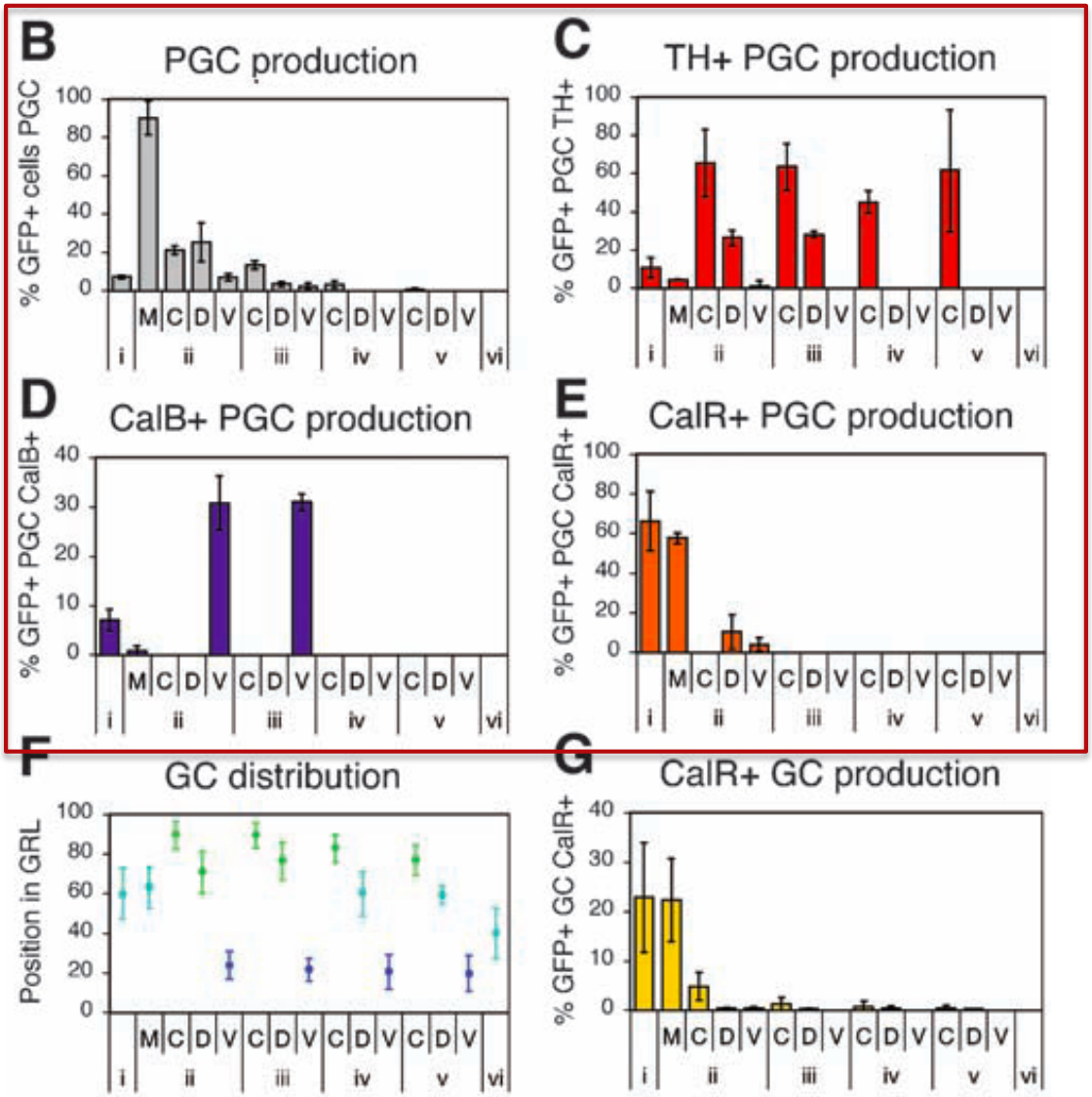
Targeting 15 different populations of radial glial cells at six different rostrocaudal levels (i to vi)



Specific target of spatially restricted radial glia by Ad-Cre and of adult SVZ stem cells they generate

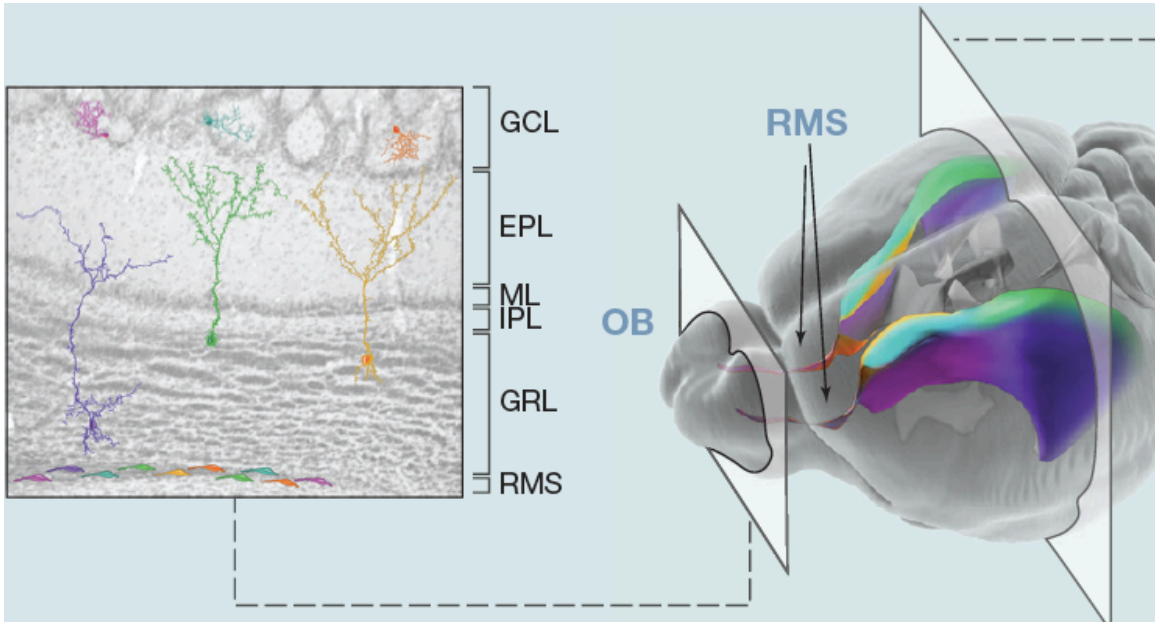


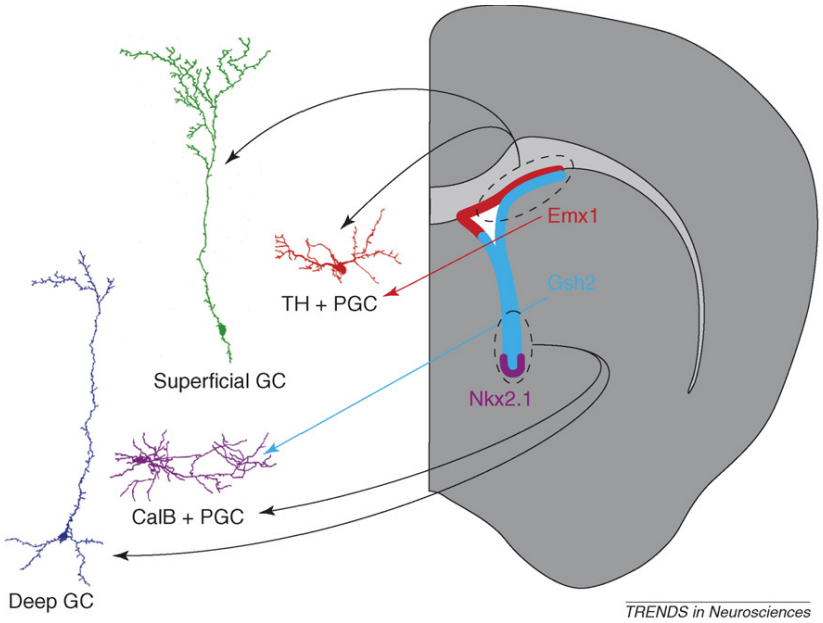




→different types of OB interneurons are derived from different locations in the SVZ

A mosaic of early specified neural stem/progenitor cells contribute to the different OB interneurons subtypes





Cheuk Ka Tong and Arturo Alvarez-Buylla
SnapShot Neuron 2014

NSC are regionally specified in both the neonatal and adult SVZ

Activity dependent regulation of Adult neurogenesis in the OB

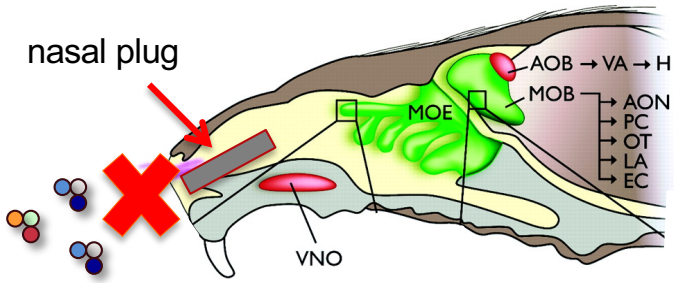
Two basic experimental paradigms to study the effect of the **environment** on **neural plasticity in the OB**

decreased adult neurogenesis

Petreanu and Alvarez-Buylla, J Neurosci 2002

Odor deprivation

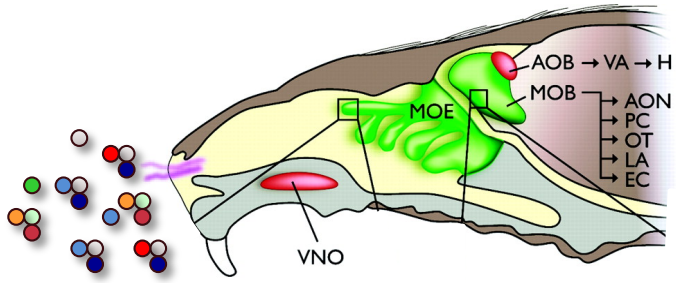
Cummings et al., 1997



decreased adult neurogenesis

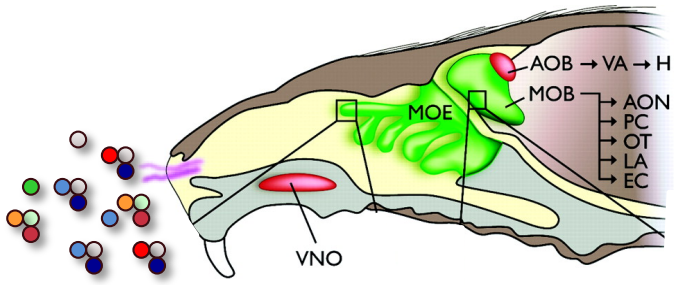
Odor enrichment

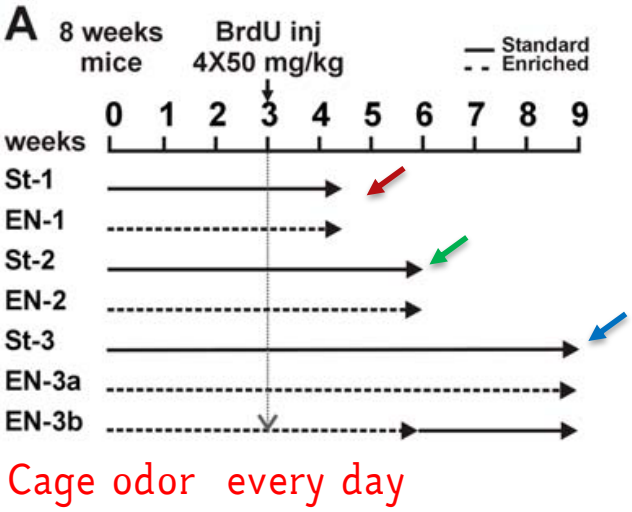
Rochefort et al., 2002



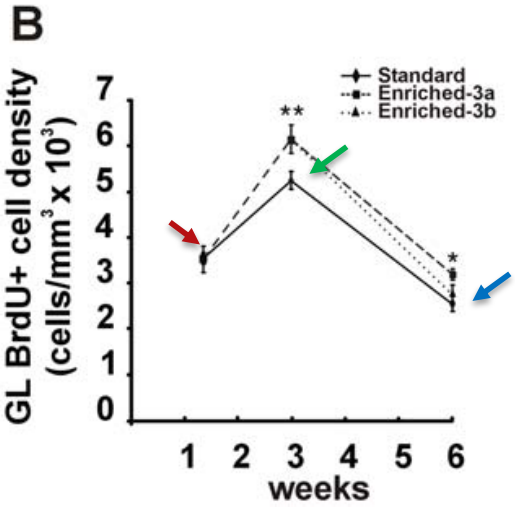
neurogenesis

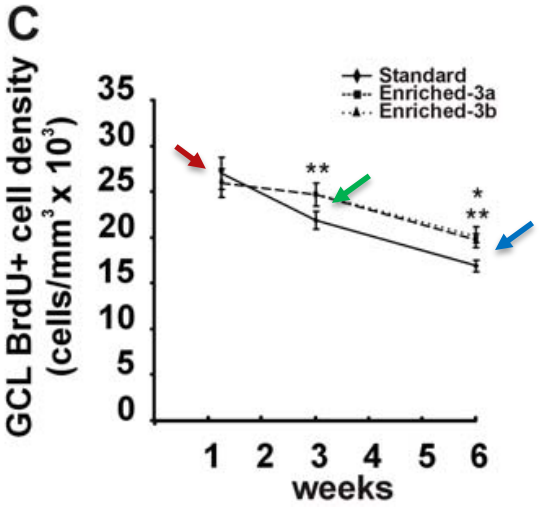
Odor enrichment





Cage odor every day

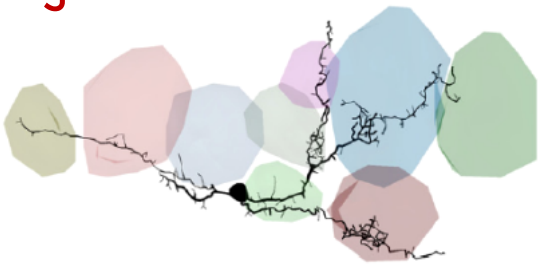


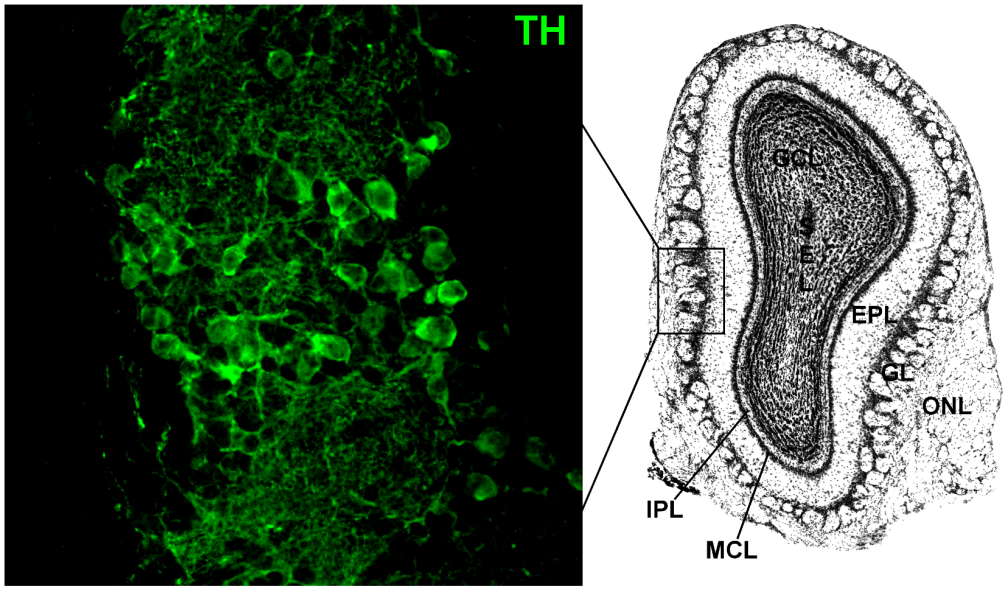


Bovetti et al., PlosOne2009

Neural plasticity in the OB: focus on TH+ dopaminergic cells

Dopaminergic interneurons





Odor deprivation by naris occlusion ⟷ Nasal plug removal

Is reversible

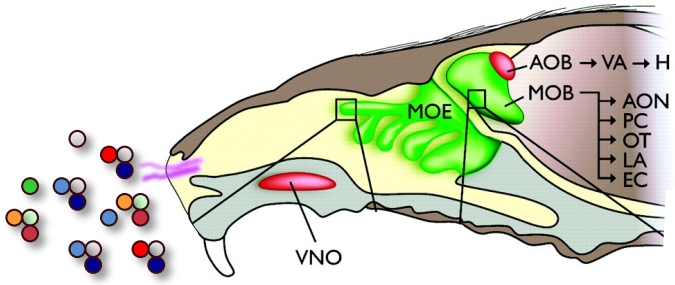
↓ decreased TH expression

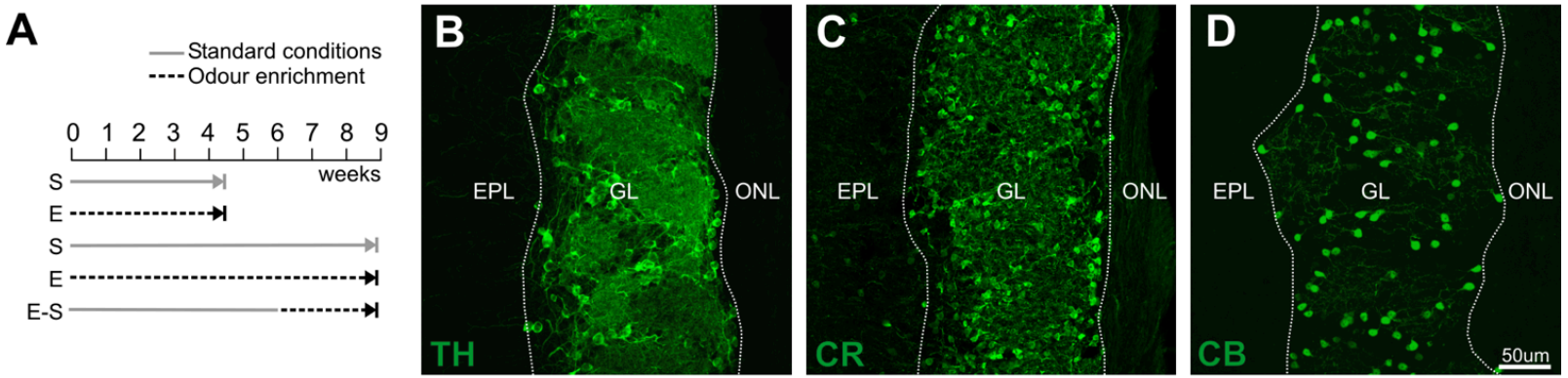
Baker et al., J Neurosci 1983
Baker et al., Brain Res. 1993

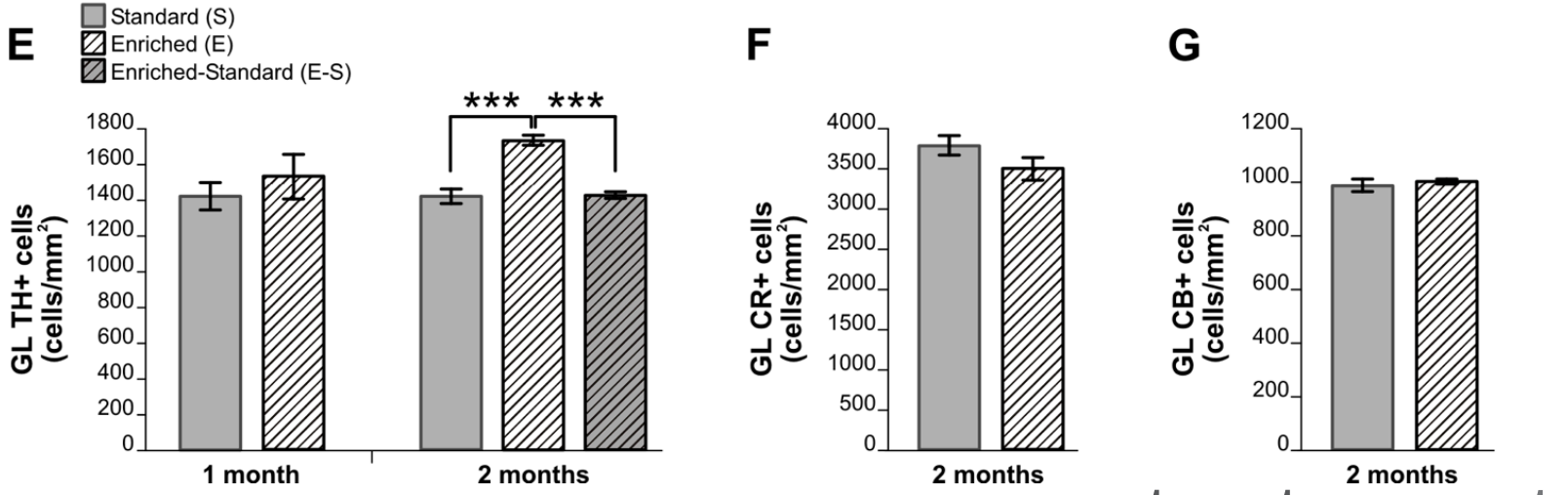
↑ TH expression

Cummings and Brunjes, J Neurosci. 1997

Odor enrichment increses TH+in the OB



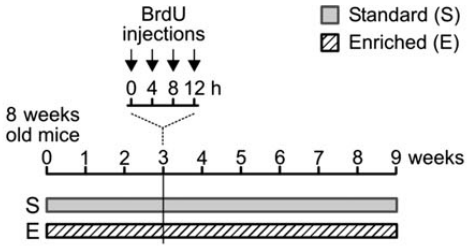


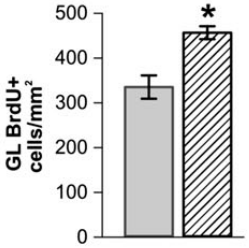


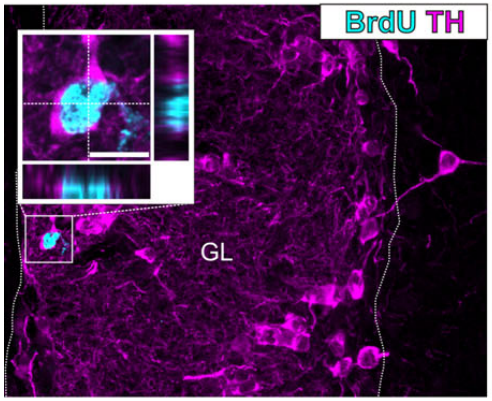
Bonzano et al., EJN 2014

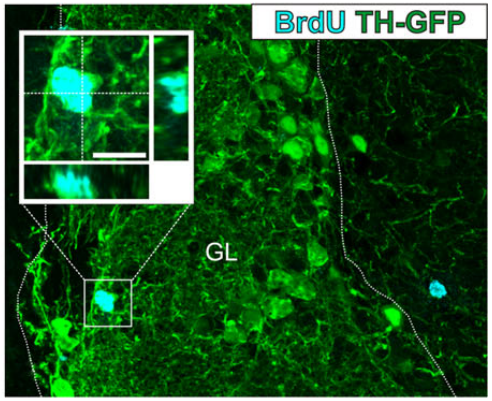
start@unito

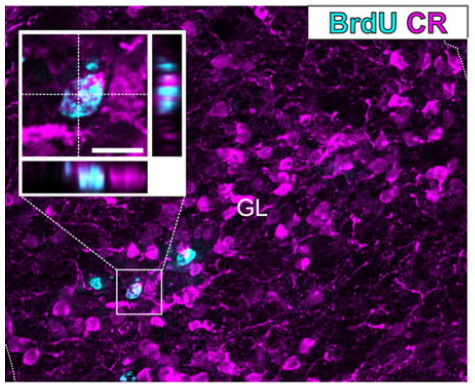
Adult neurogenesis underlies TH+ cell increase following odor enrichment

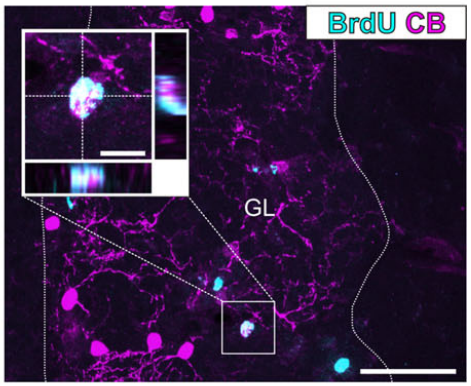


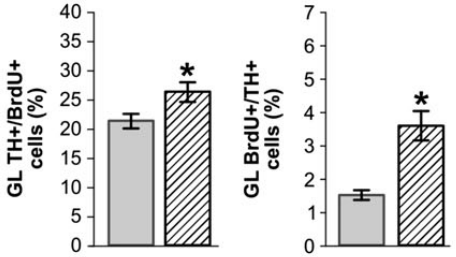


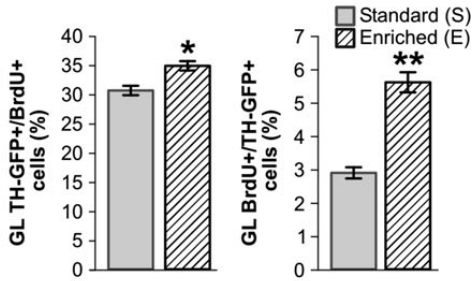


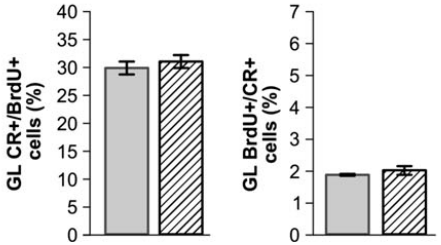


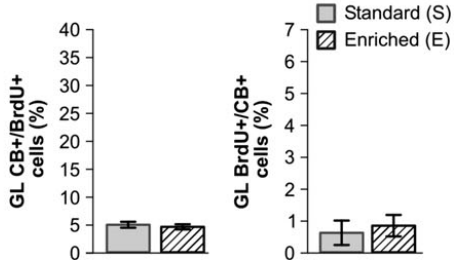








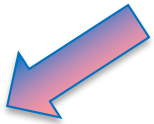




Generation of specific OB interneurons subtypes

Cell intrinsic mechanisms

Cell extrinsic cues



Selective survival of OB interneurons subtypes