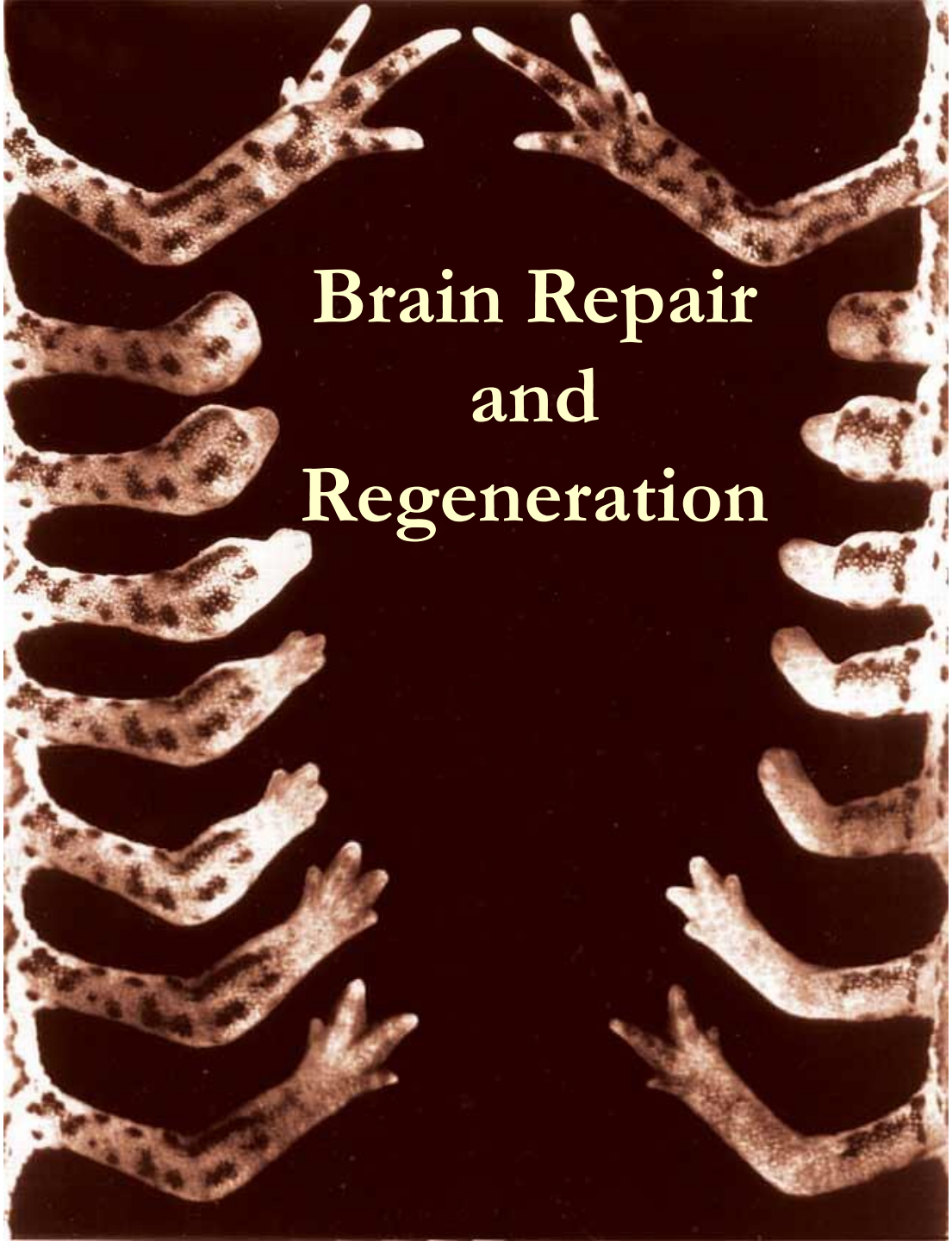

Brain Repair
and
Regeneration

Causes of Neuronal Degeneration

Acute Degeneration

Progressive Degeneration

Causes of Neuronal Degeneration

Acute Degeneration

Infection

Stroke (ictus)

Traumatic injuries

….

Progressive Degeneration

Neurodegenerative diseases

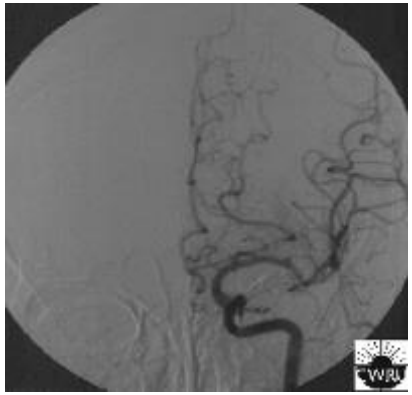
(Parkinson, Huntington, Alzheimer,
Amyotrophic lateral sclerosis..)

Stroke (ictus)

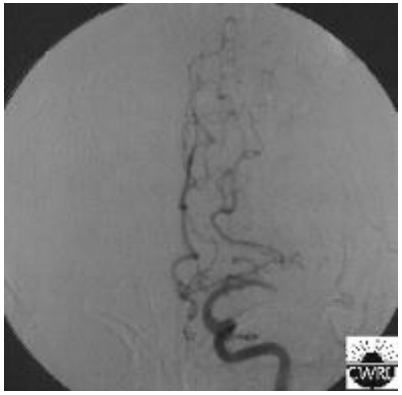
Primary cause of disability (USA ed EU)
Secondary Cause of death (world wide)

POOR BLOOD FLOW TO THE CNS RESULTS IN CELL DEATH

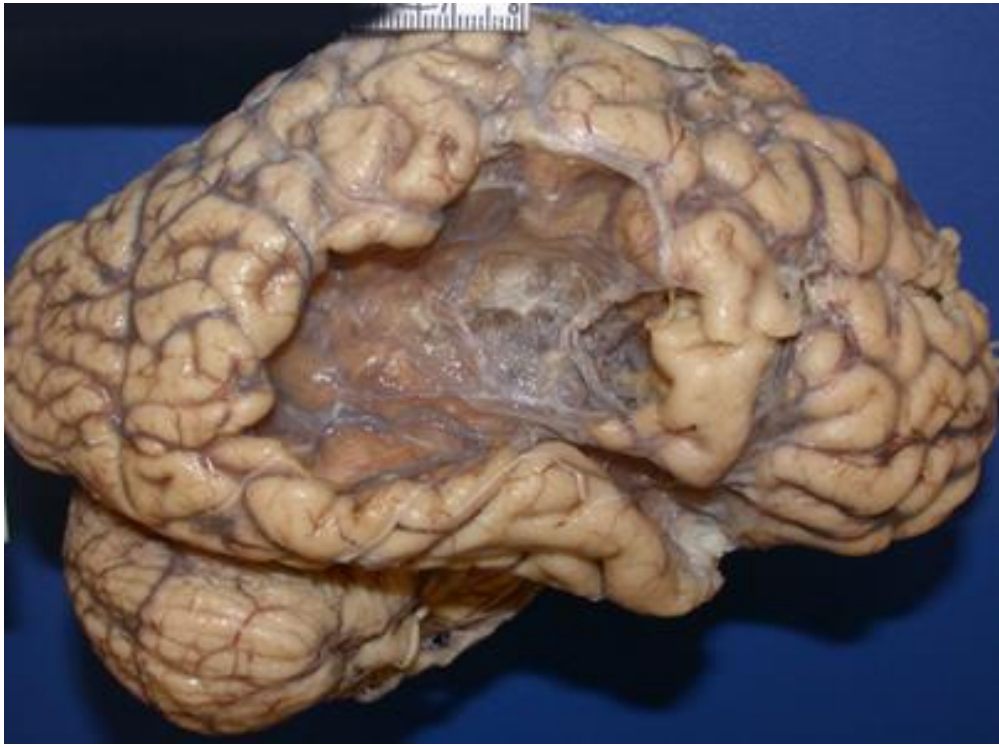
Ischemic stroke (85%)
Haemorrhagic stroke(15%)



Normal middle cerebral artery

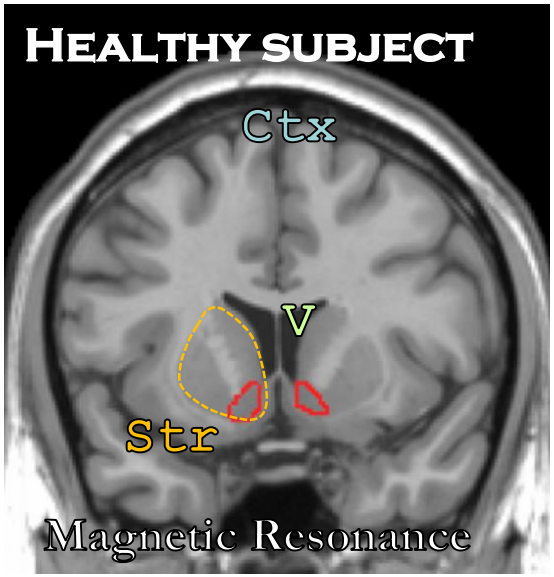


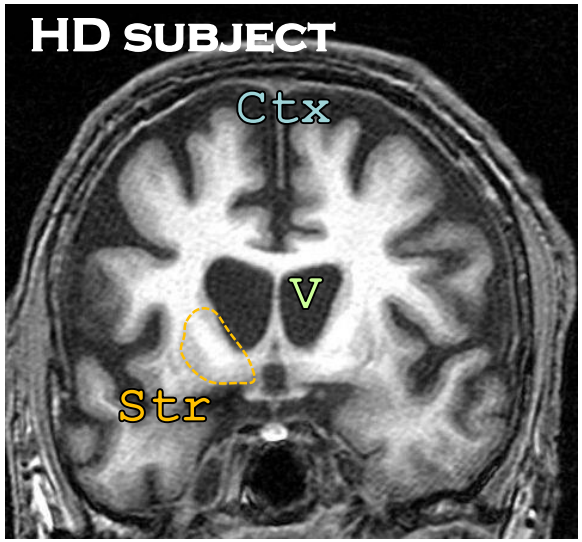
Bloked middle cerebral artery



The Right Hemisphere of a patient after an event of stroke involving the middle cerebral artery

Huntington's disease





Str: striatum
V: ventricle
Ctx: cortex

Huntington's disease is a genetic neurodegenerative disorder (polyglutamine diseases)

The HD is caused by the expansion of a normally occurring CAG triplet repeat within the coding region of the huntingtin gene.

That results in an expanded glutamine tract in the protein, causing a pathological gain-of-function of the huntingtin protein.

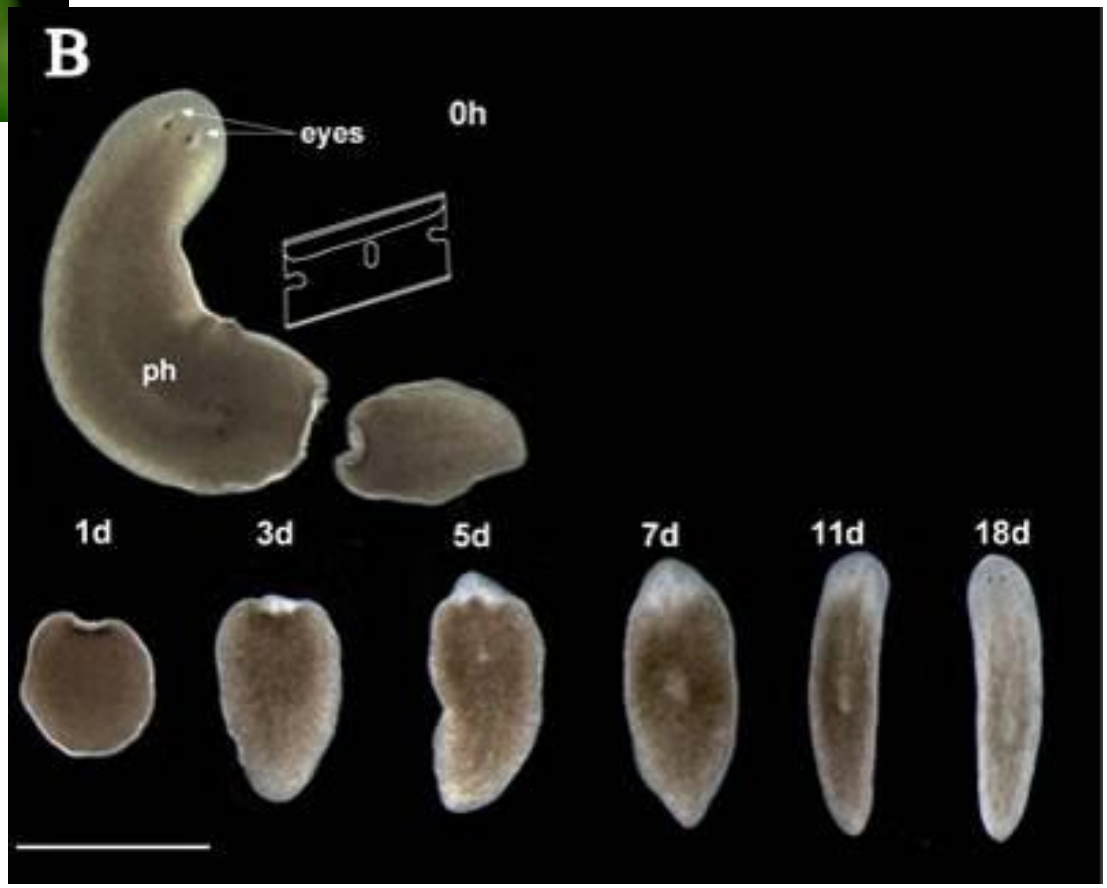
The striatum is the primary site of pathology. Strong degeneration of the striatal projection neurons (medium spiny neurons)
Other brain regions, such as the neocortex, undergo neuronal degeneration

HD results in involuntary choreic movements (Greek for "dance" uncontrollable jerking movements), as well as dementia and psychiatric dysfunction

GIULIO BIZZOZERO CLASSIFICATION OF TISSUES

LABILE	STABLE	EVERLASTING
Continous renewal Regenerative capacity	Minimum renewal Regenerative capacity	No renewal No regenerative capacity
EPIDERMIS	LIVER	SNC

Regeneration in planarian

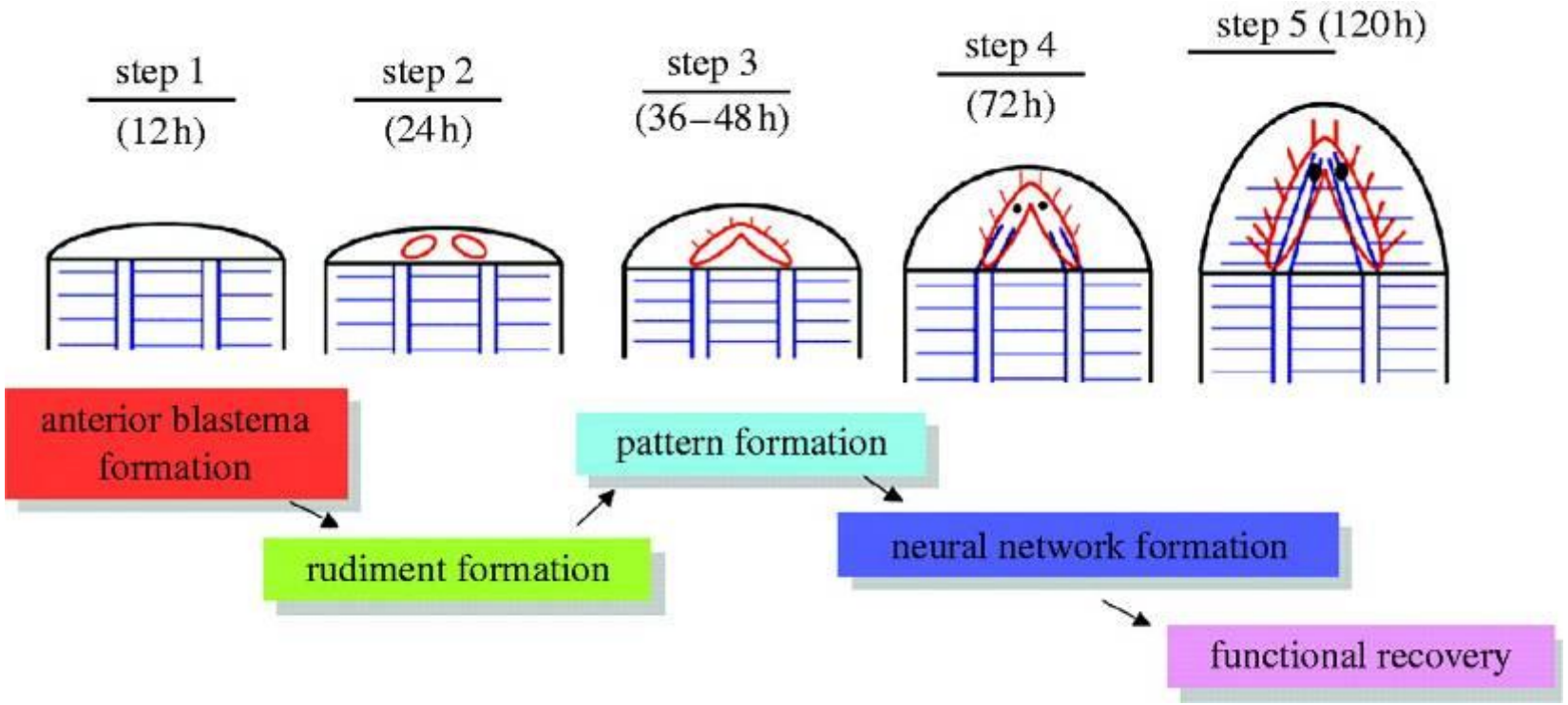


Planarians can regenerate an entire body including the brain from a small piece of the body in which no brain tissues remain.

Regeneration of the entire brain

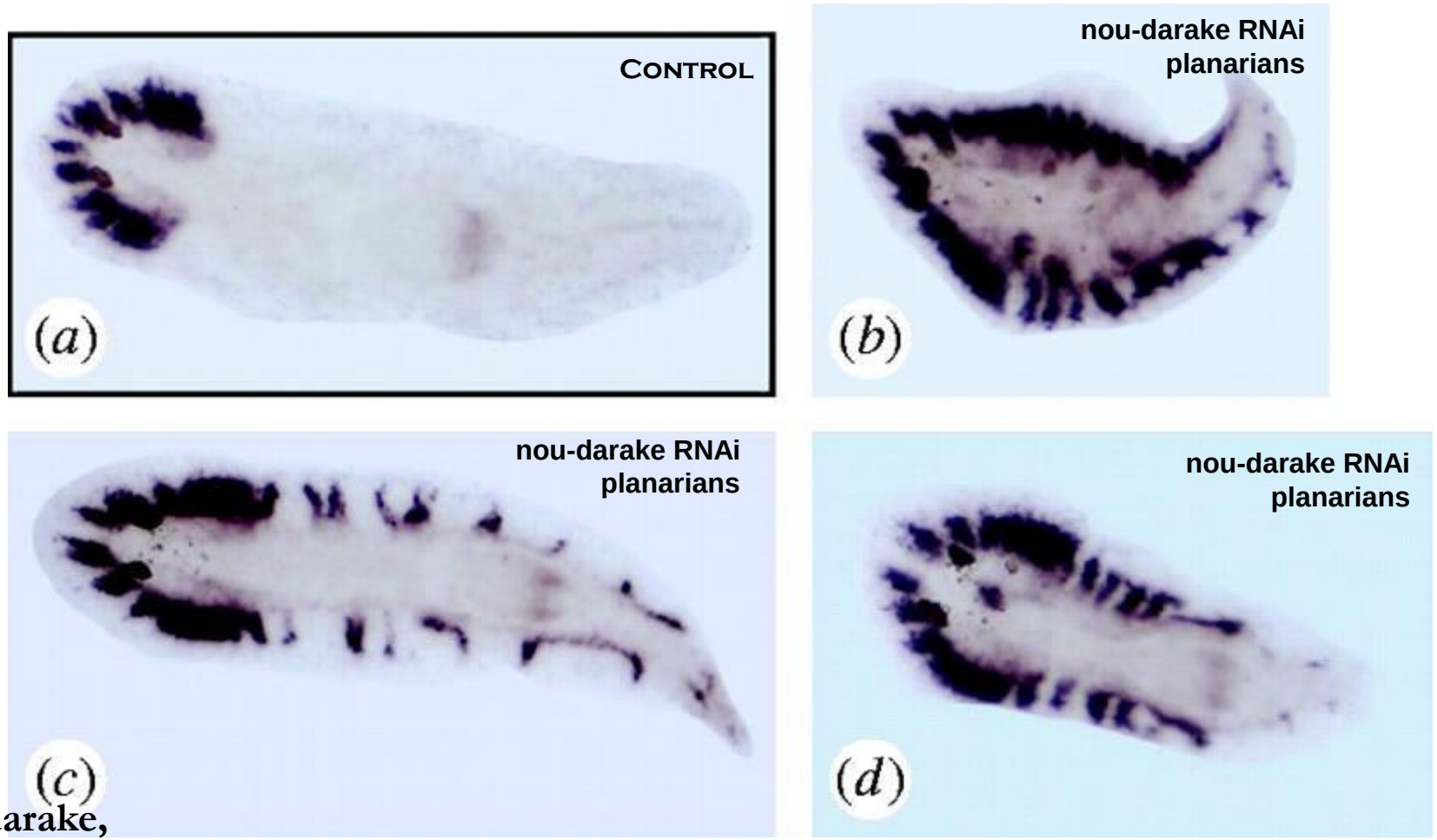
Pluripotent stem cells are activated and form a blastema

They repeats the embryonic development steps



Agata K , Umesono Y Phil. Trans. R. Soc. B 2008;363:2071-2078

The planarian maintains continously active signals that regionalize the embryo. By interfiring with the activity of a morphogen (FGF) in nou-darake RNAi planarians →Brains are ectopically formed in all regions of the body

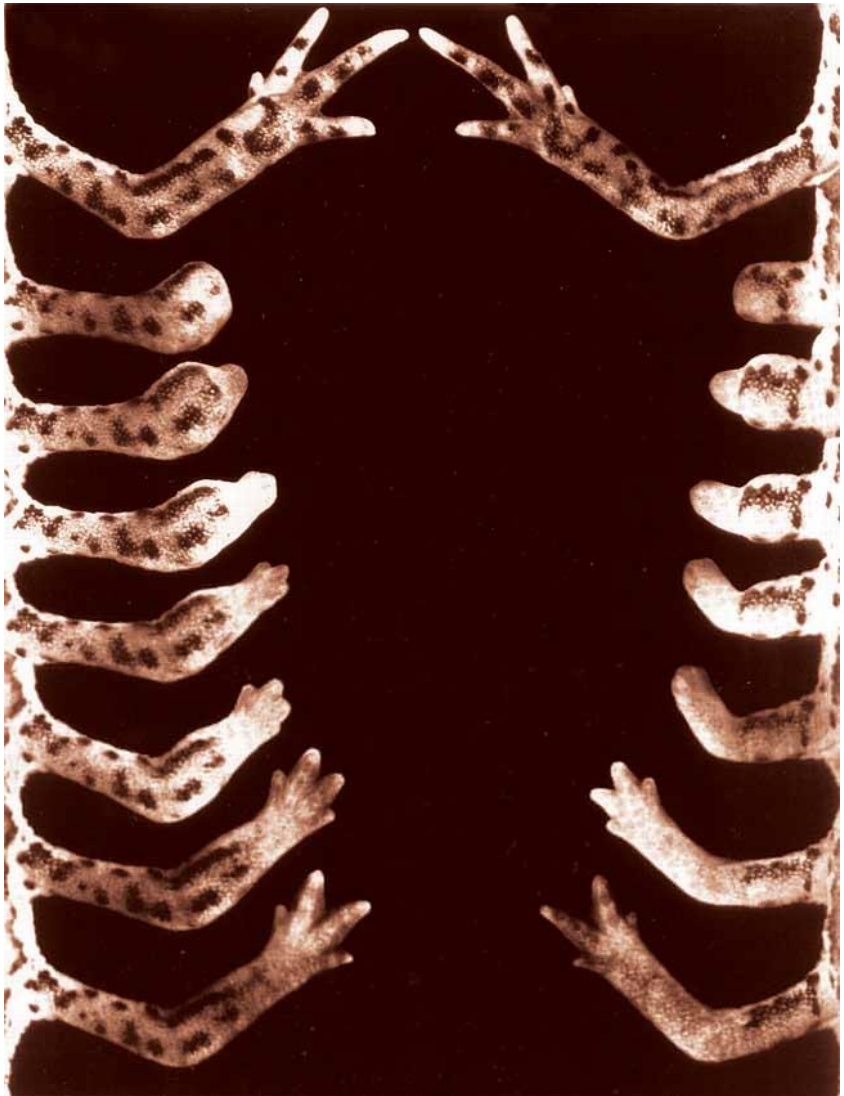


nou-darake, 'brains everywhere' in Japanese

Agata K , Umesono Y Phil. Trans. R. Soc. B 2008;363:2071-2078

HOWEVER, SUCH EVENTS ARE MUCH RARER IN THE VERTEBRATES..

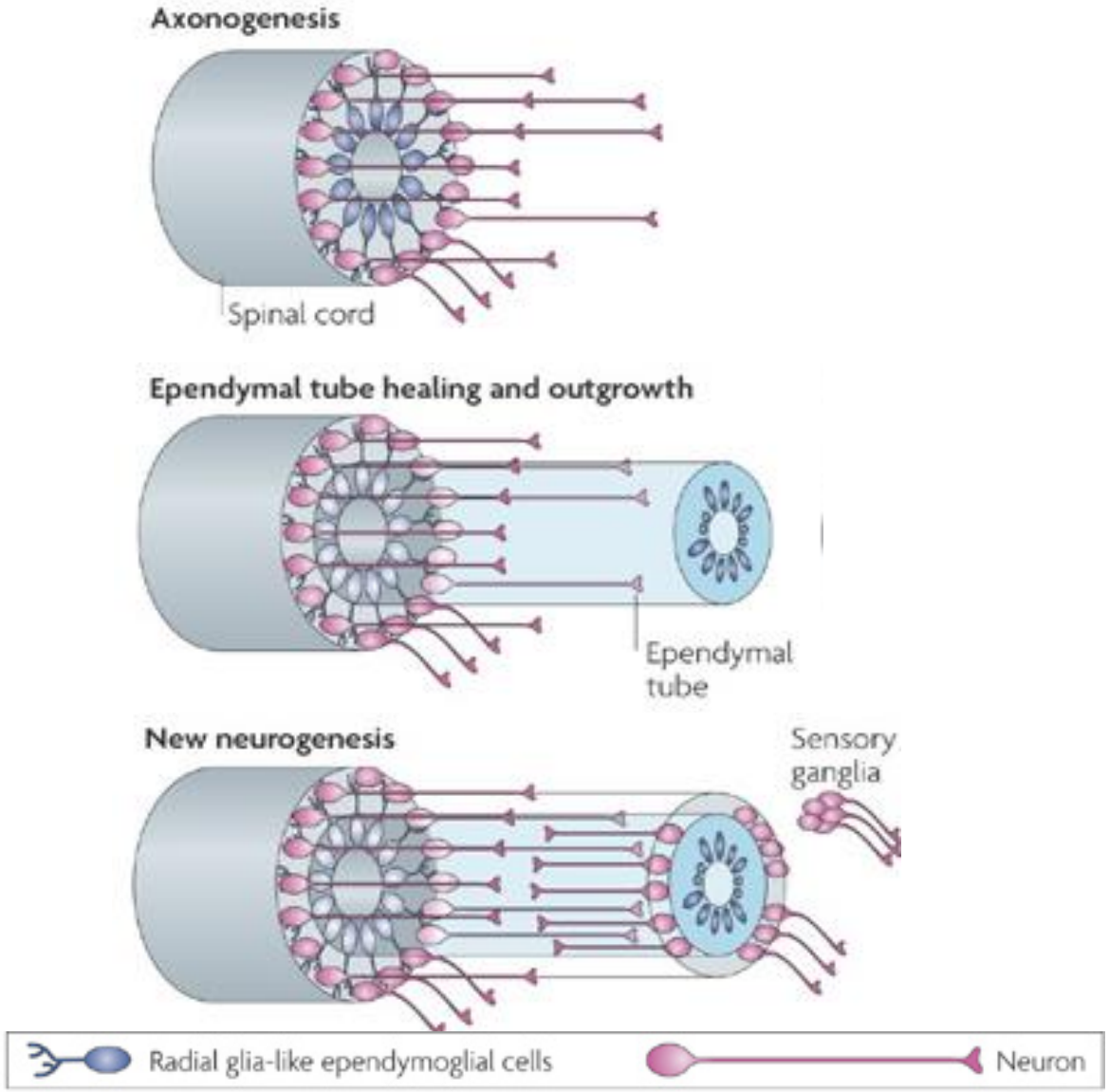
Among these there are some salamanders (urodeles) , for example the Axolotls , which can regenerate whole parts of the body .



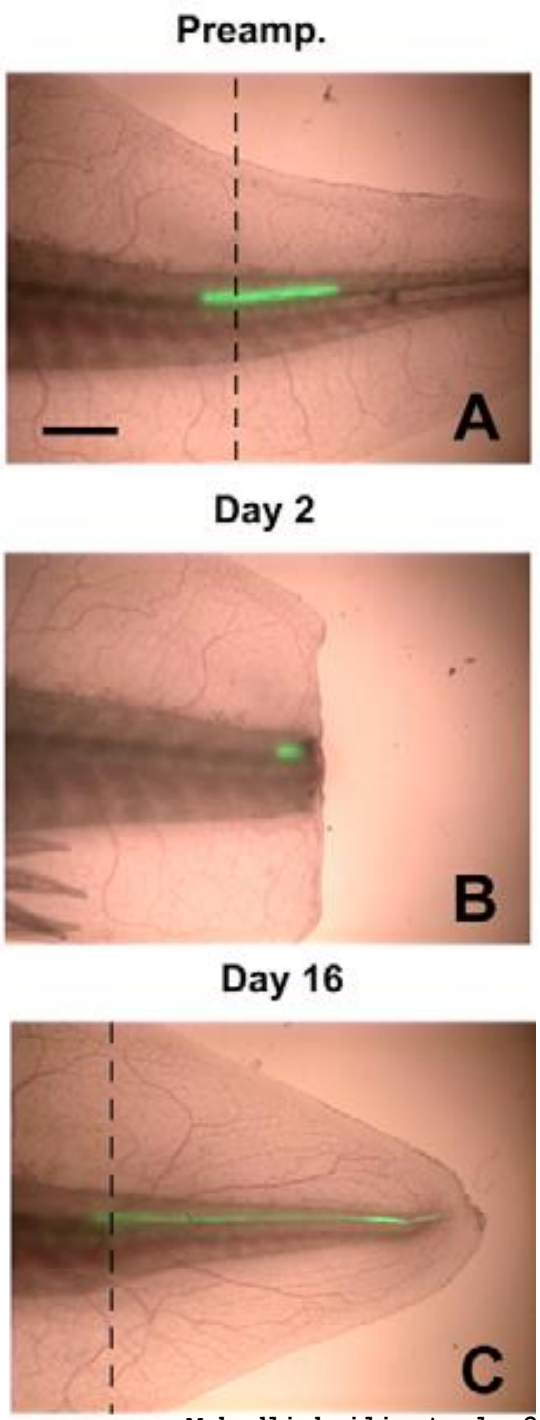
Paedomorphosis: retention of larval traits in the adult life. **Neoteny,** they reach sexual maturity without undergoing metamorphosis

Spinal Cord Regeneration in Axolotl

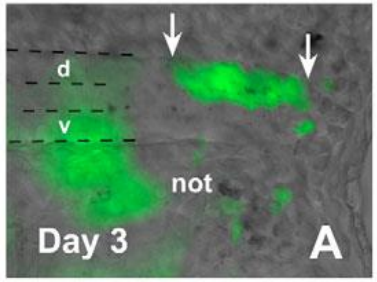
(Neotenic animal)

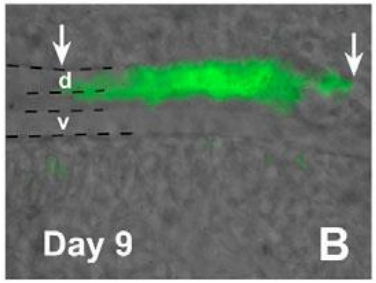


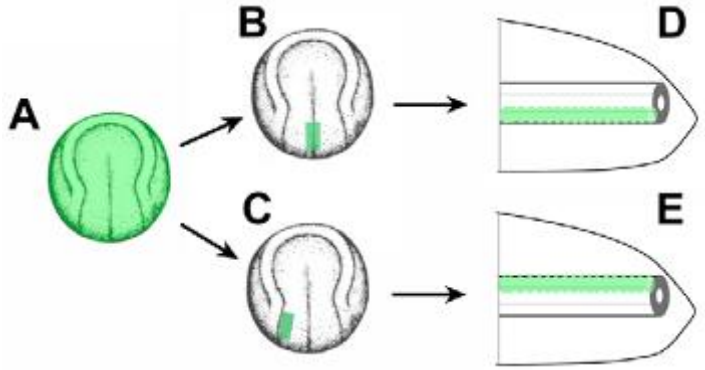
Tanaka and Ferretti 2009

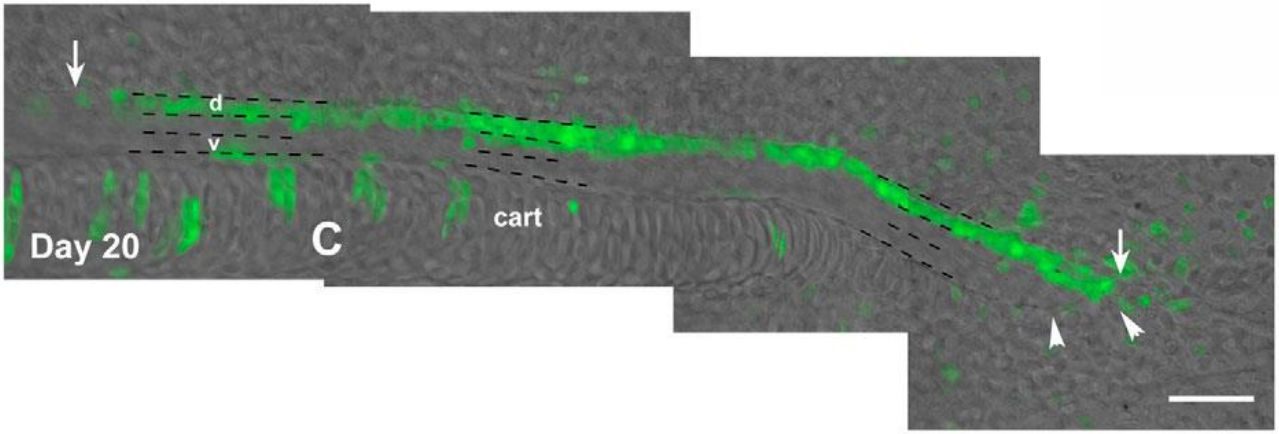


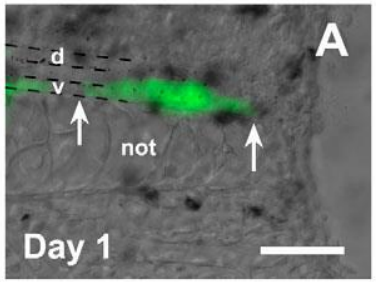
Mchedlishvili et al. 2007

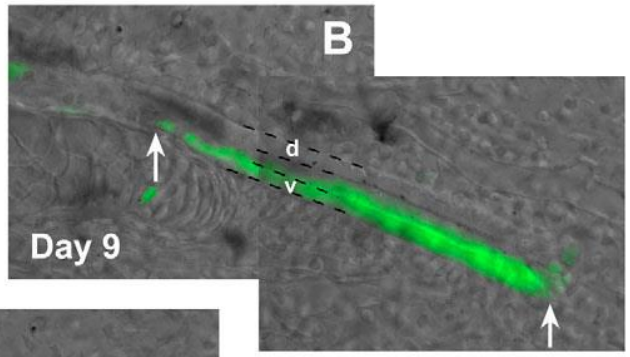


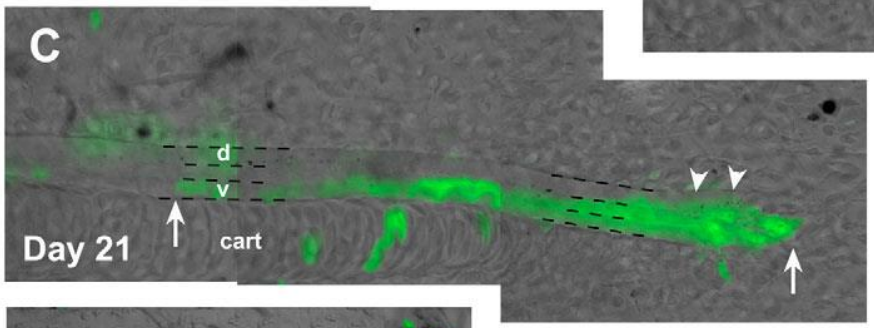










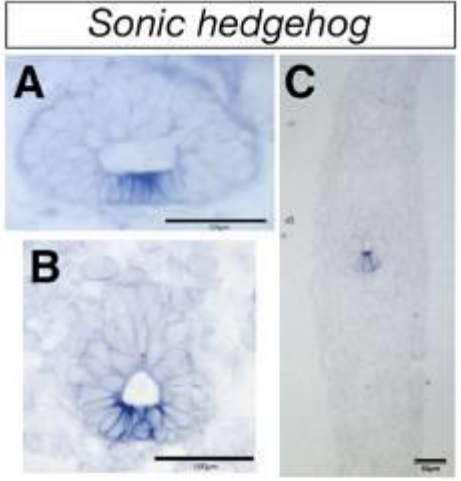


Dorsal spinal cord transplant

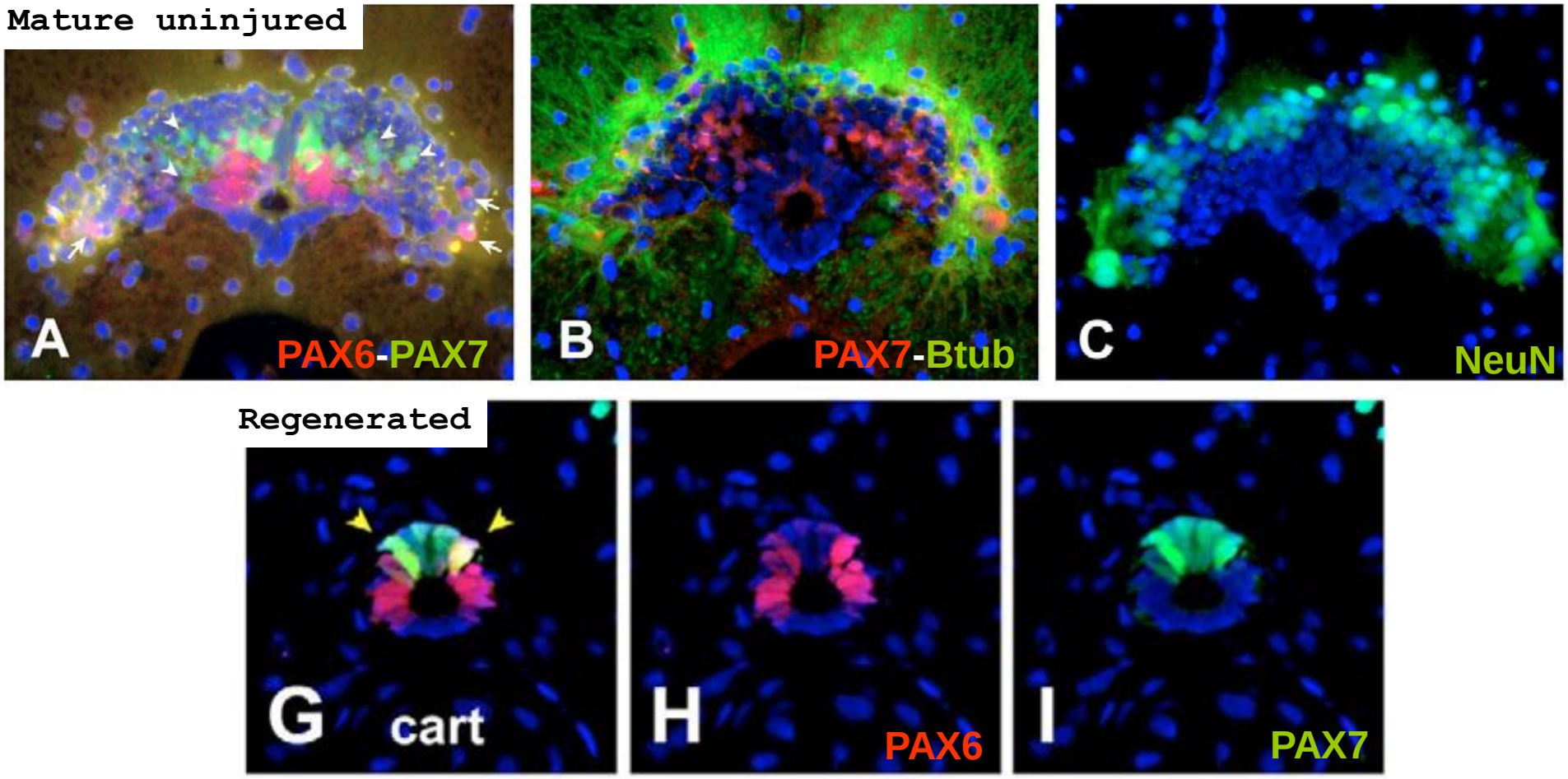
Ventral spinal cord transplant

eGFP+ tissue were removed from prospective posterior ventral or dorsal neural tube regions of germline eGFP transgenic embryos and grafted into white hosts. The tail was amputated in the adult life.

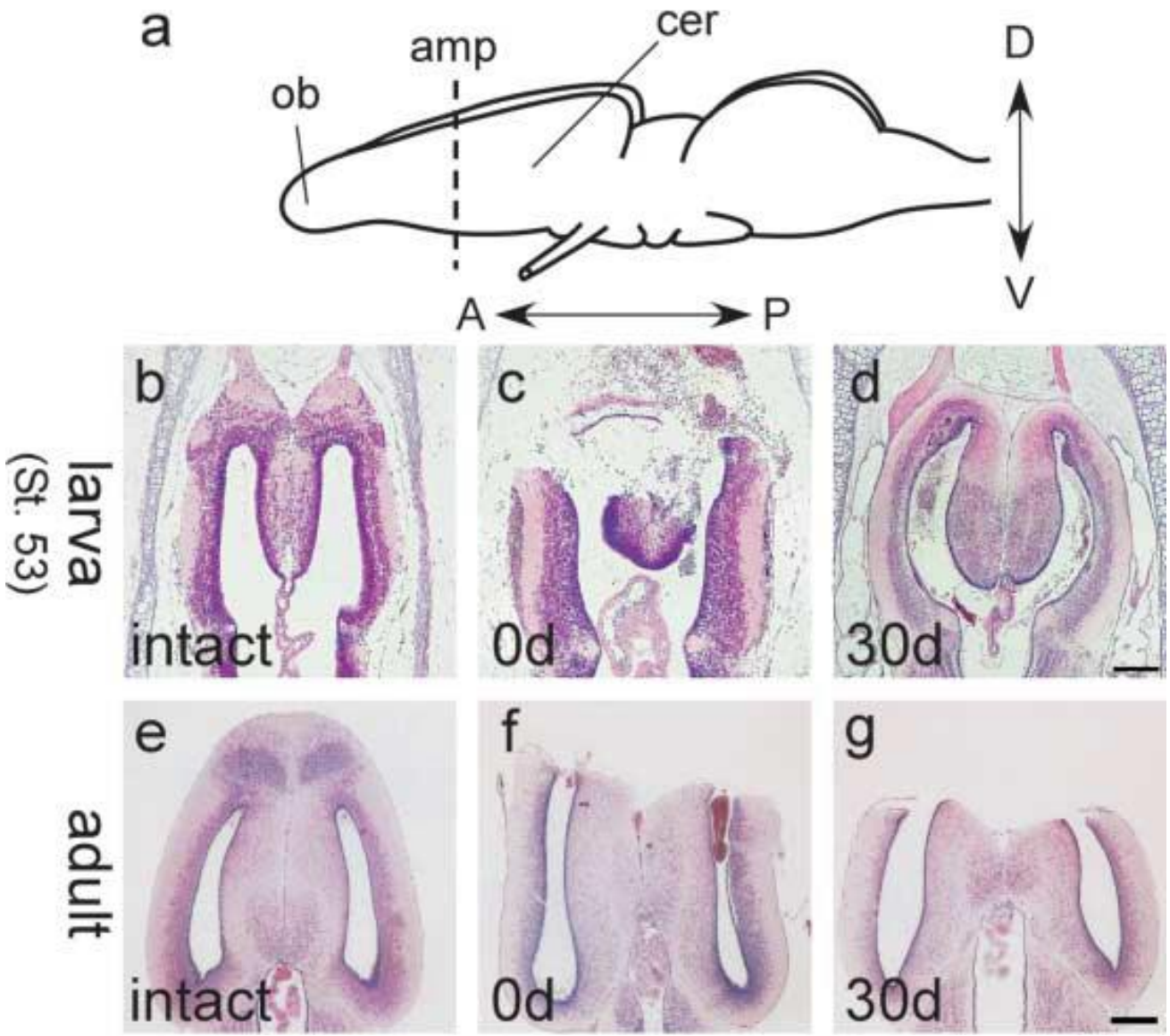
Green cells give rise to cells with the same dorsoventral position.



The ventricular glial progenitors retain the expression of morphogens and genes specific to the embryonic stage also during adult life .
These cells are then able to direct the formation of different neuronal subtypes also post lesion.



The anurans amphibians show capacity for regeneration. However only during the larval stage

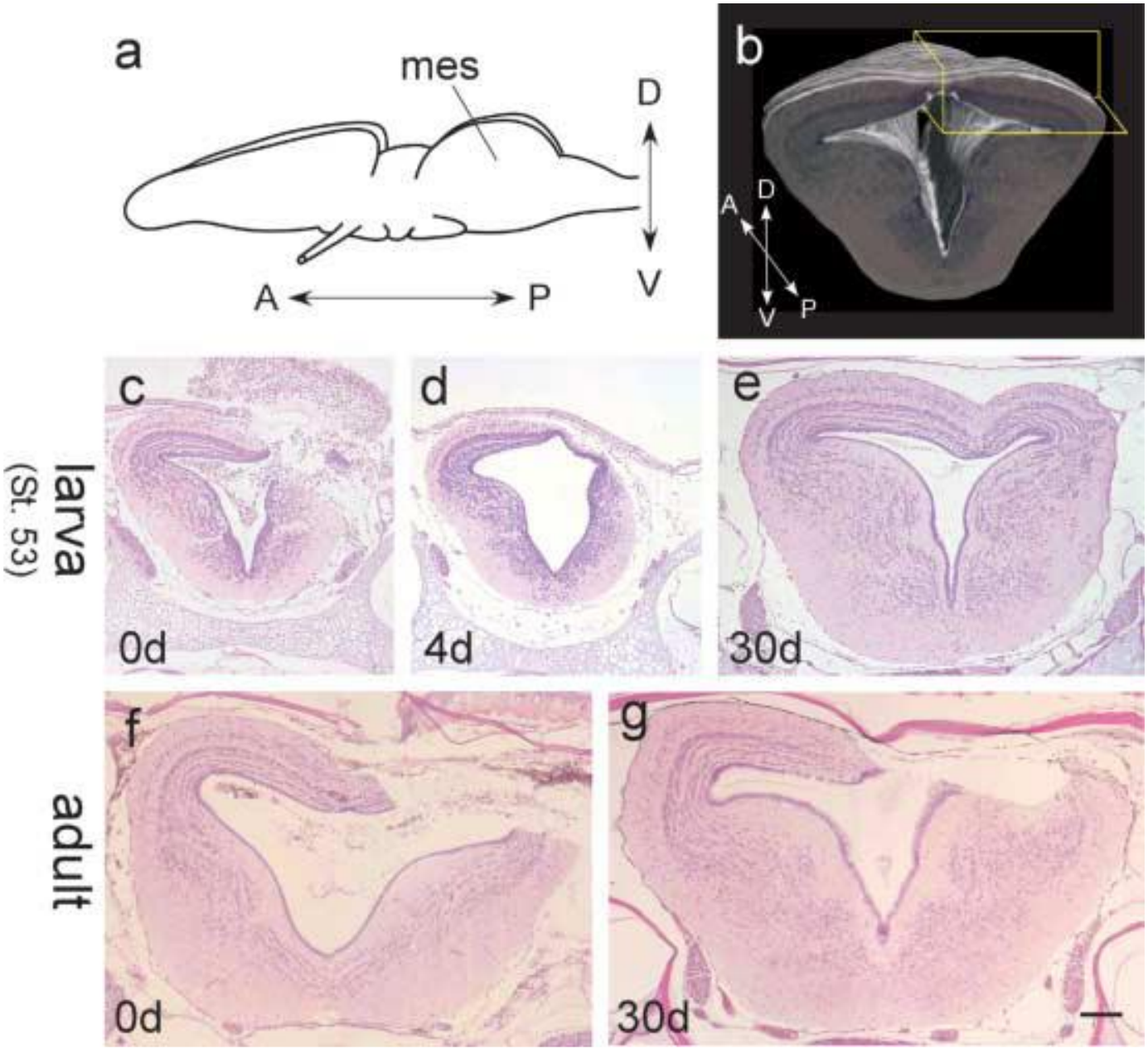


Regeneration of the anterior telencephalon and the olfactory bulbs

Note : Even in mammals and birds remarkable regenerative capabilities are displayed during embryonic life.

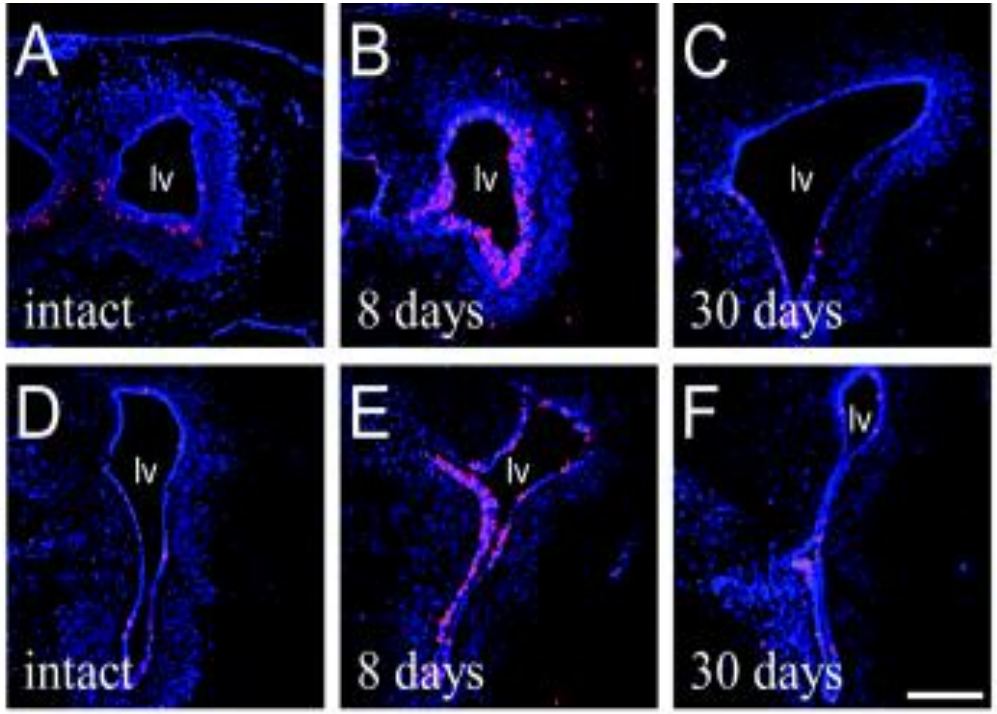
Yoshino et al., 2004

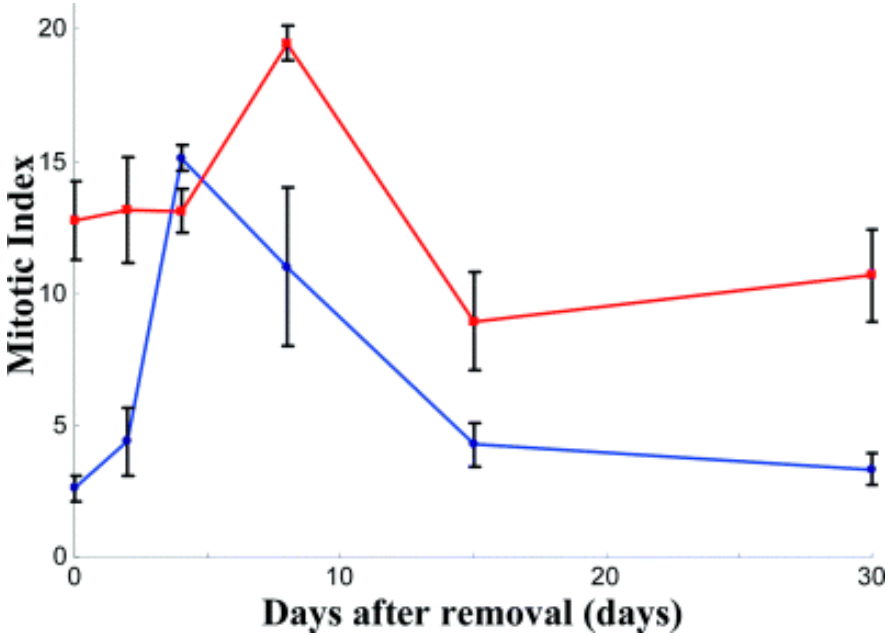
Regeneration of the optic tectum



larva

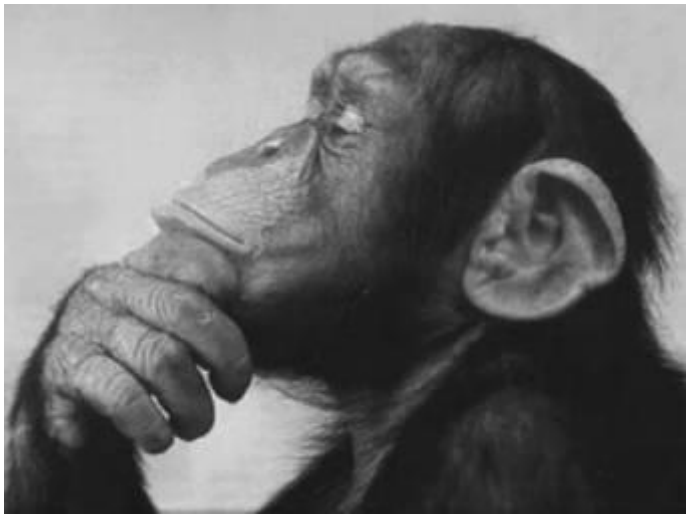
Froglet





The cells of the VZ respond to the lesion with an increased proliferation both before (larva) and after metamorphosis (Froglet).

Even in mammals display remarkable regenerative capabilities.
However, only during embryonic stages



Note: in adult mammals :

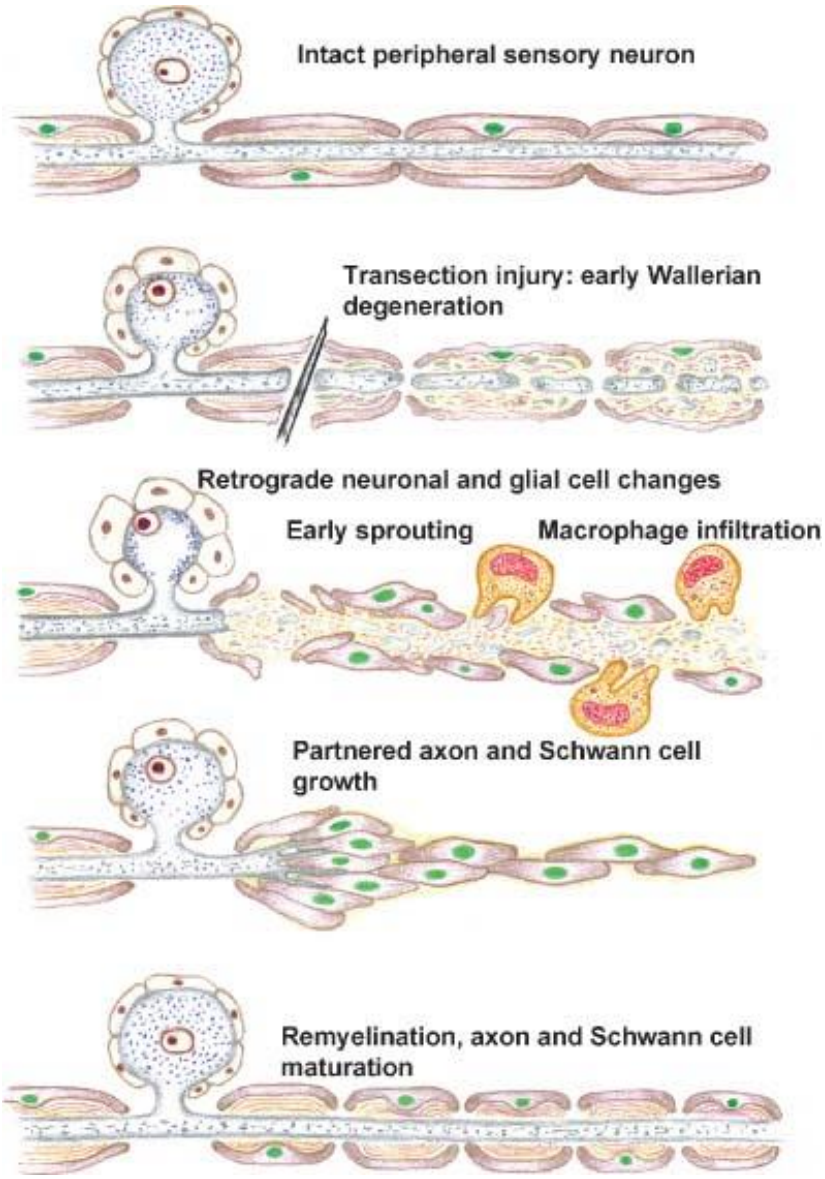
the peripheral nervous system (PNS)

the central nervous system (CNS)

display different regenerative capacity

The peripheral nervous system (PNS)

The PNS possesses the potential for regeneration



Key role:

- **Glia → cellule di Schwann**
- **macrophages**

Limiting Factor

Distance

The central nervous system (CNS)

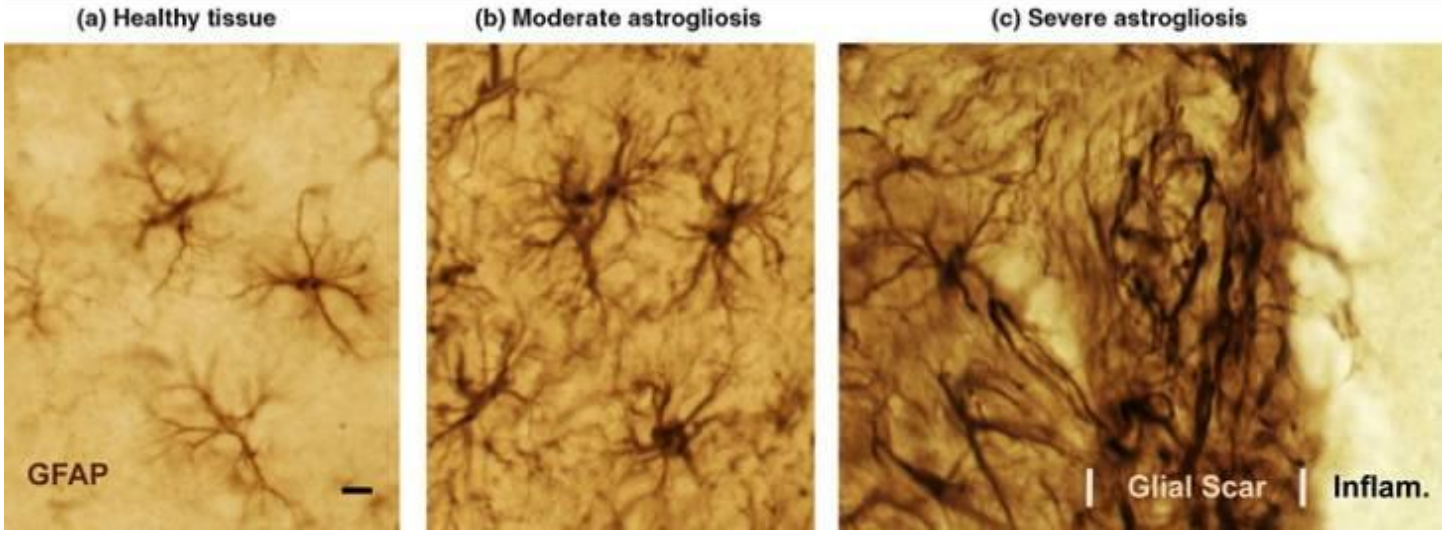
The CNS loses the ability to regenerate during adulthood. Why?

Limiting Factors:

- **Environment:** no expression of essential growht factors
- **Slow degradation of the distal stump**

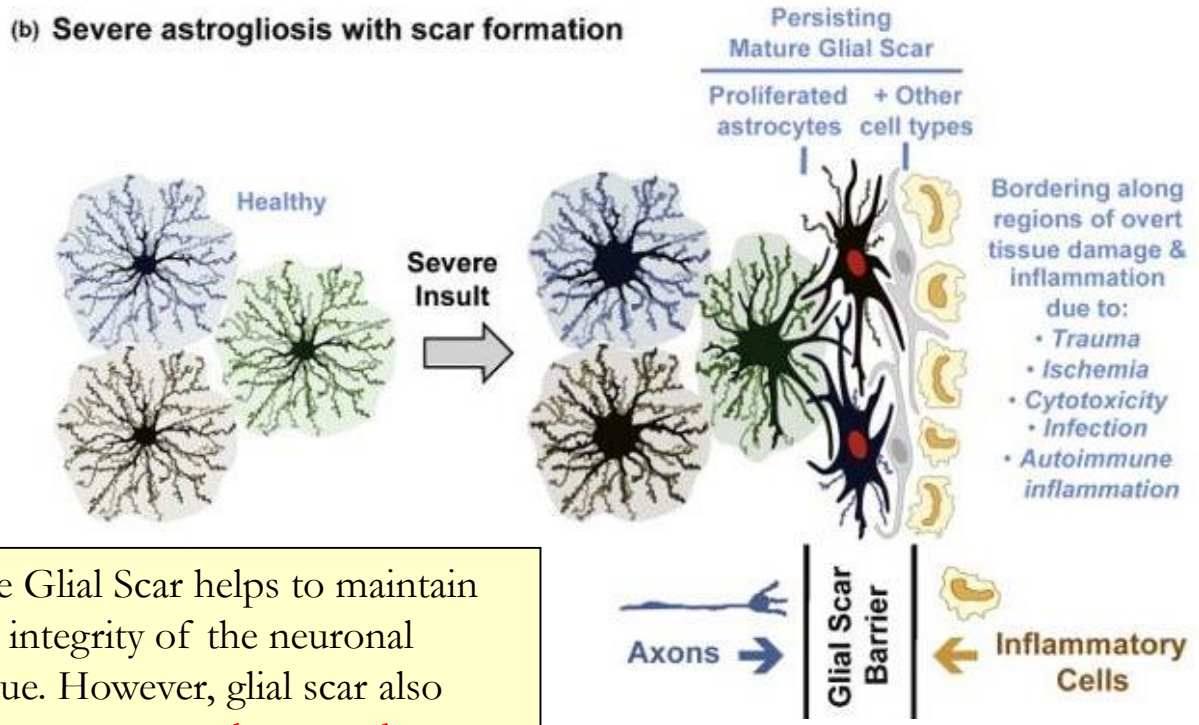
- Glia: **Astrocytes**
  - → Reactive astrogliosis
  - → Glial scar

Reactive astrogliosis and glial scar formation



Following tissue damage, astrocytes become hypertrophic, begin to proliferate.

In severe cases, they form a glial scar that forms a barrier for cells and external molecules but also for the axons.



The Glial Scar helps to maintain the integrity of the neuronal tissue. However, glial scar also prevents neuronal regrowth.

Sofroniew MV 2009

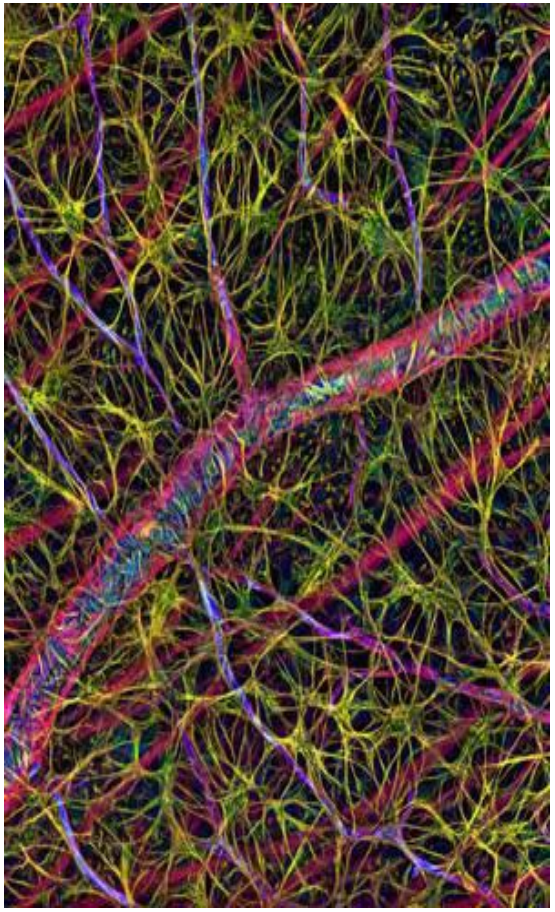
ASTROCYTES

This type of glial cell is missing in other vertebrates

PHYSIOLOGICAL FUNTIONS:

Regulate blood flow in the brain
Metabolic support
Glycogen fuel reserve buffer
Maintenance of extracellular ion balance
Neurotrasmitter uptake and release
Modulation of synaptic transmission

Some of them… act as **neural progenitors**



In adult mammals , **LESIONS** strongly stimulate the **ASTROCYTE PROLIFERATION**

Even the astrocytes that act as neural progenitors increase their proliferation after injury..

Thus, lesions stimulate the proliferation of

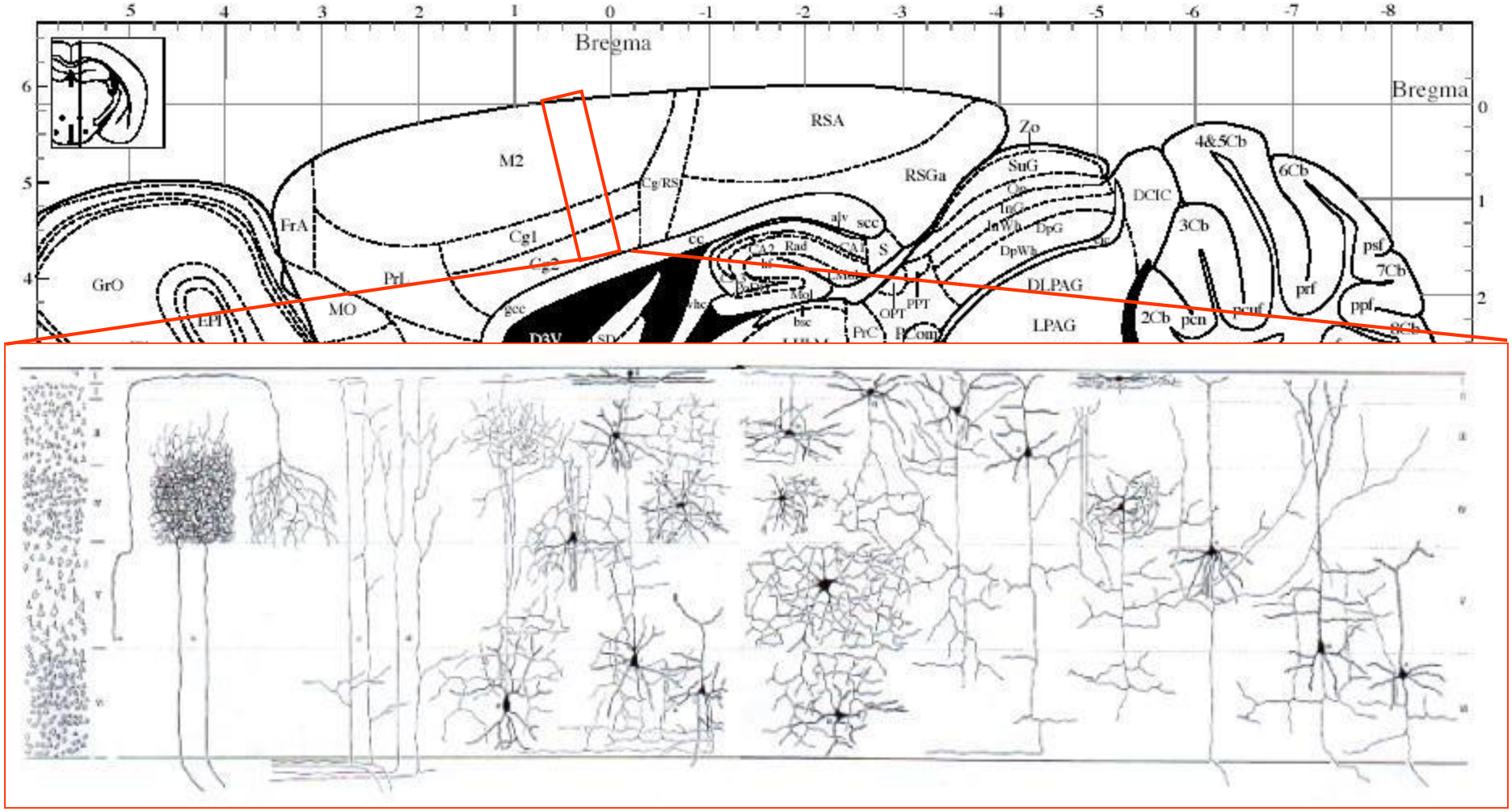
ADULT NEURAL STEM CELLS

What is the neurogenic potential of the mammalian adult brain?

How many types of neurons can be generated?

Neuronal diversity in the central nervous system

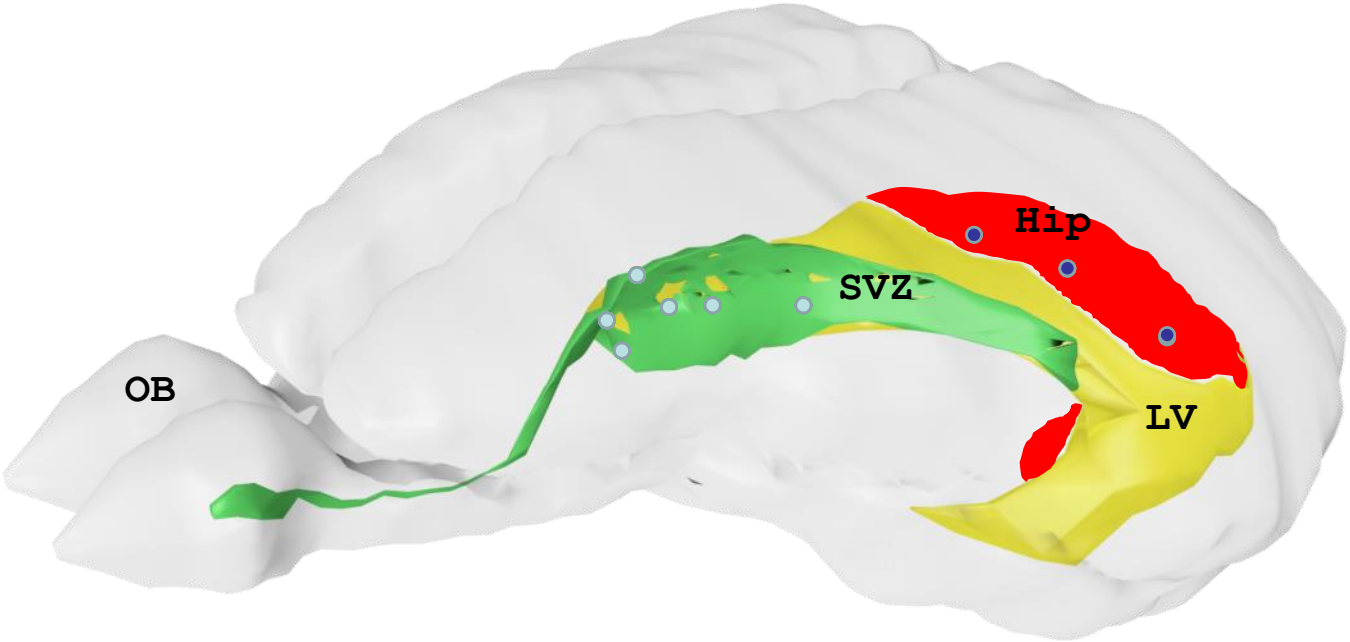
Each area → huge diversity of neurons!!!



Adult neural stem cells give rise to all neuronal types?

NO!!

- **SVZ→** olfactory bulb interneurons
- **SGZ →** dentate gyrus granule cells



Adult neural progenitors give rise to specific subpopulations of neurons

Comparative studies …

have shown that in some mammalian species, including humans, low level of neurogenesis can also occur in regions normally non-neurogenic in mice, such as the:

Striatum (rabbit, guinea pig, non human primates, human)

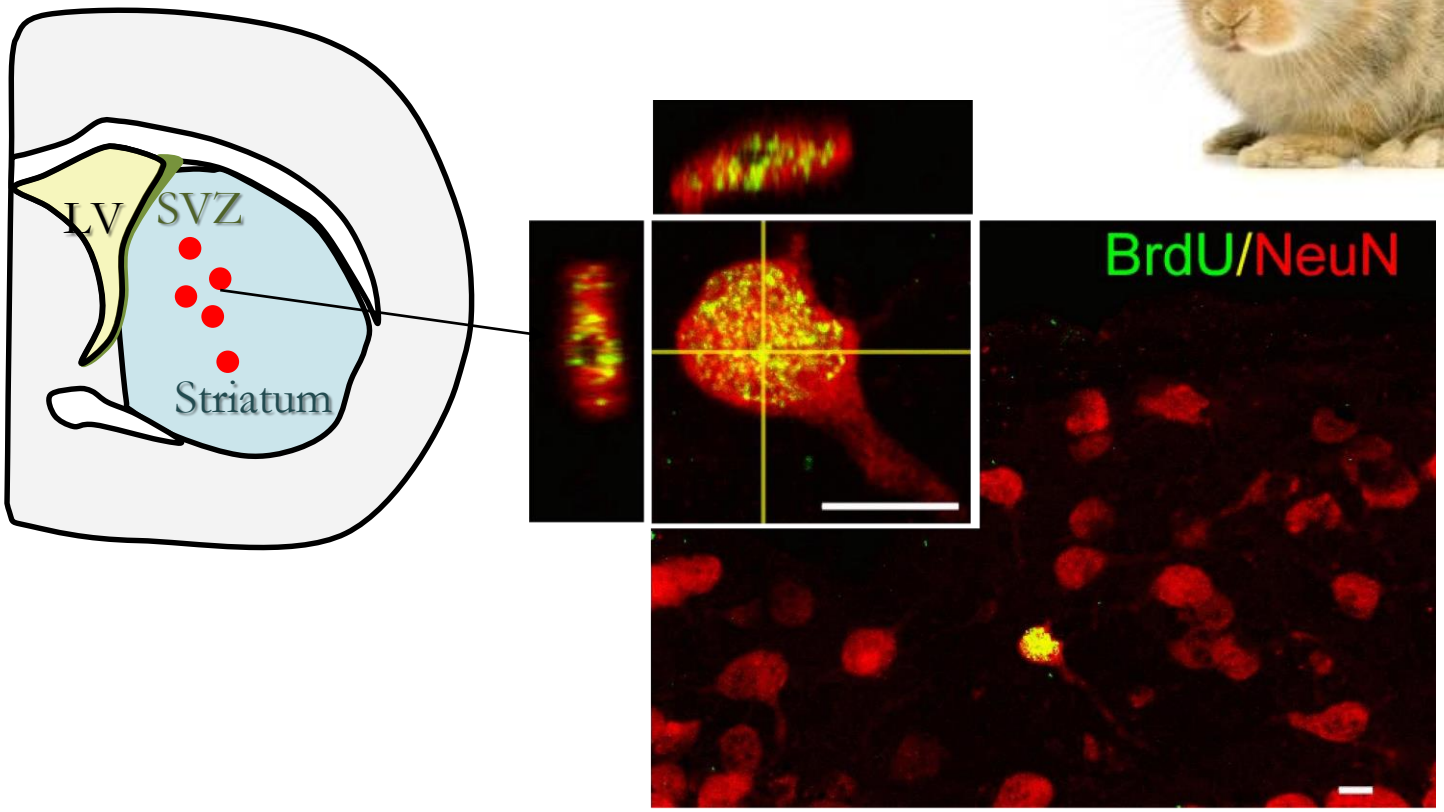
Neocortex (non human primates, rabbit)

Amygdala (non human primates, prairie vole)





Adult neurogenesis in the rabbit striatum

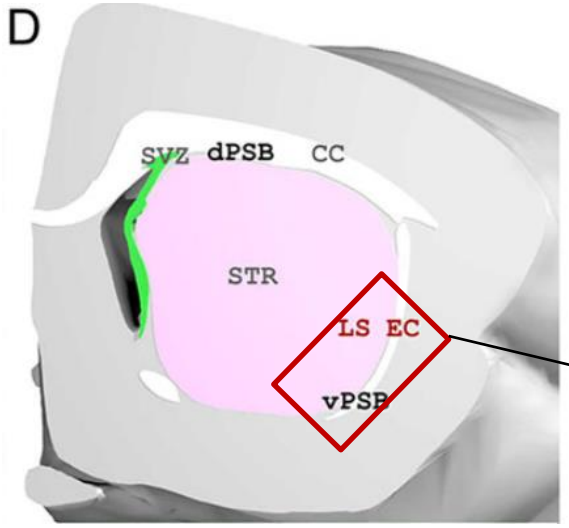


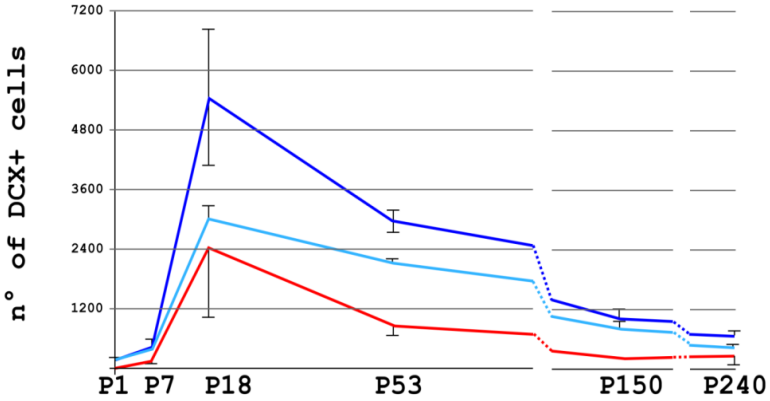
Newly generated neurons in the adult rabbit striatum

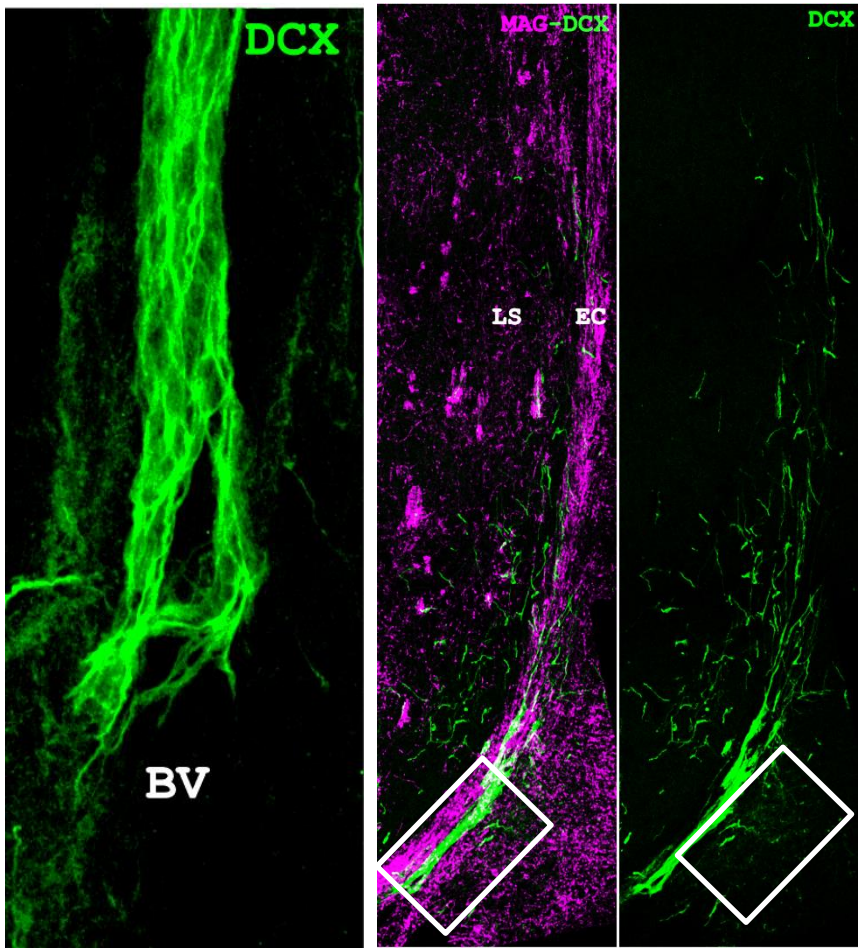
BrdU proliferation marker
NeuN mature neurons marker

Luzzati et al, 2006

Adult neurogenesis in the Guinea pig



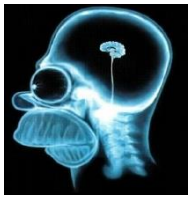


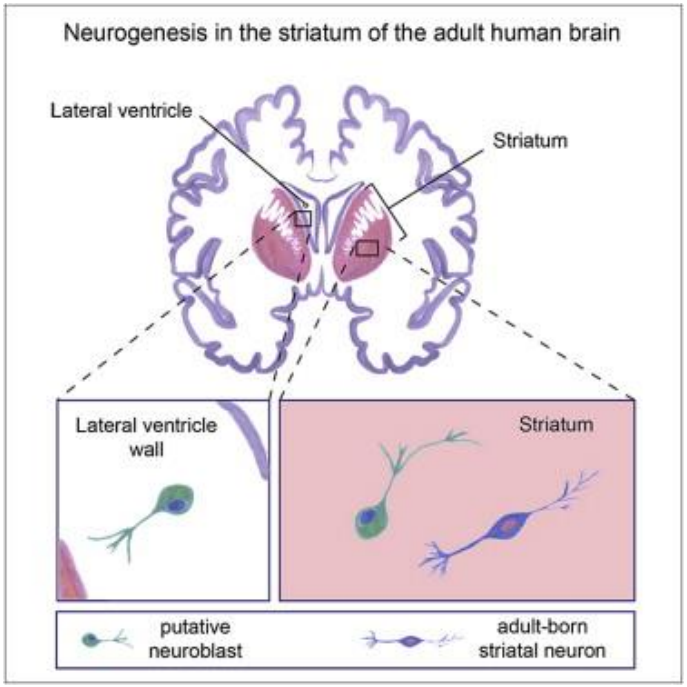


DCX marker of immature neurons

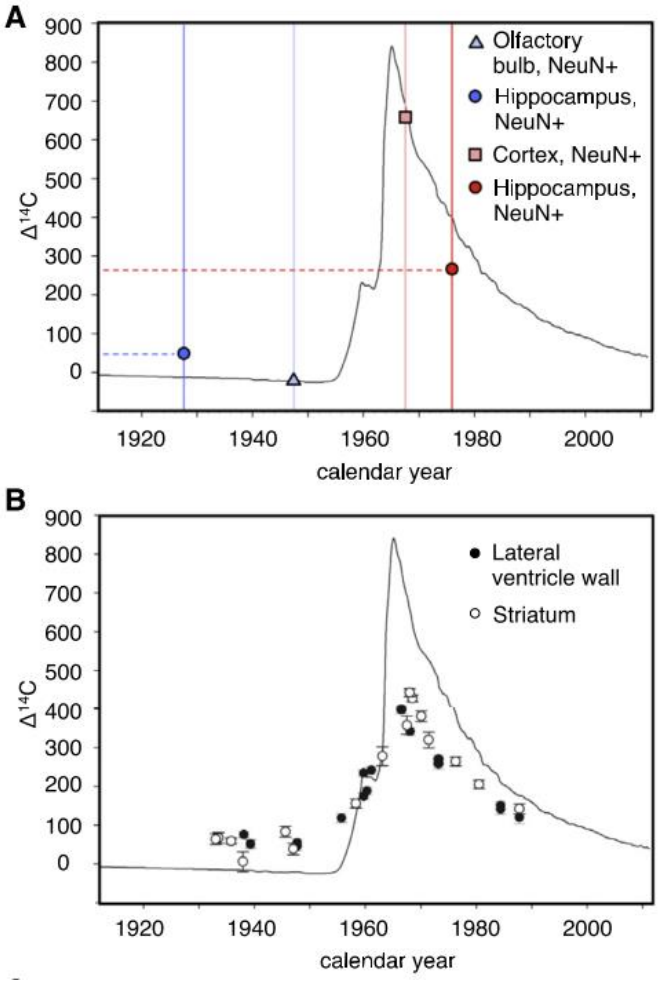
Luzzati et al, 2014

Adult neurogenesis in the human striatum

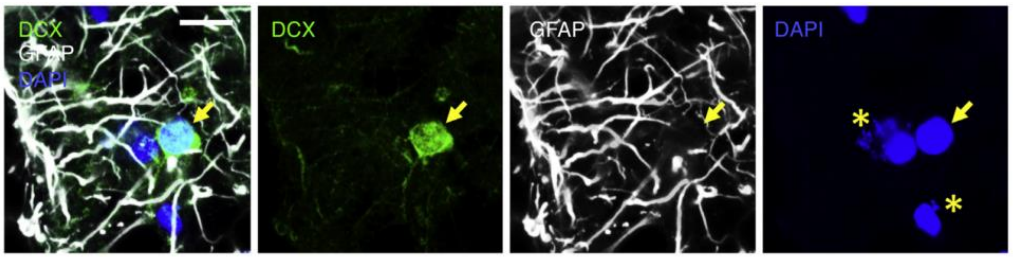




Retrospective Birth Dating of Striatal Cells revealed continuous generation of striatal interneurons



DCX+ neuron in the human striatum



Ernst, 2014

Comparative studies demonstrated that adult neurogenesis can also occur in regions normally non-neurogenic in mice

In addition, it has been shown that brain lesions can induce neurogenesis in these same regions also in laboratory rodents

PATHOLOGIC CONDITION

Striatum (mice, rat)

Neocortex (mice, rat)

Hippocampal CA1 (mice, rat)

Open questions

What is the relevance of these findings in term of fate potential?

- What is the fate and the role of the newborn neurons?

- What is the identity of the progenitors activated after lesion?

  •Constitutively active adult NSCs have a broader cell fate potential

  OR

  •Neural progenitors that are quiescent in physiological condition and become active after lesions?

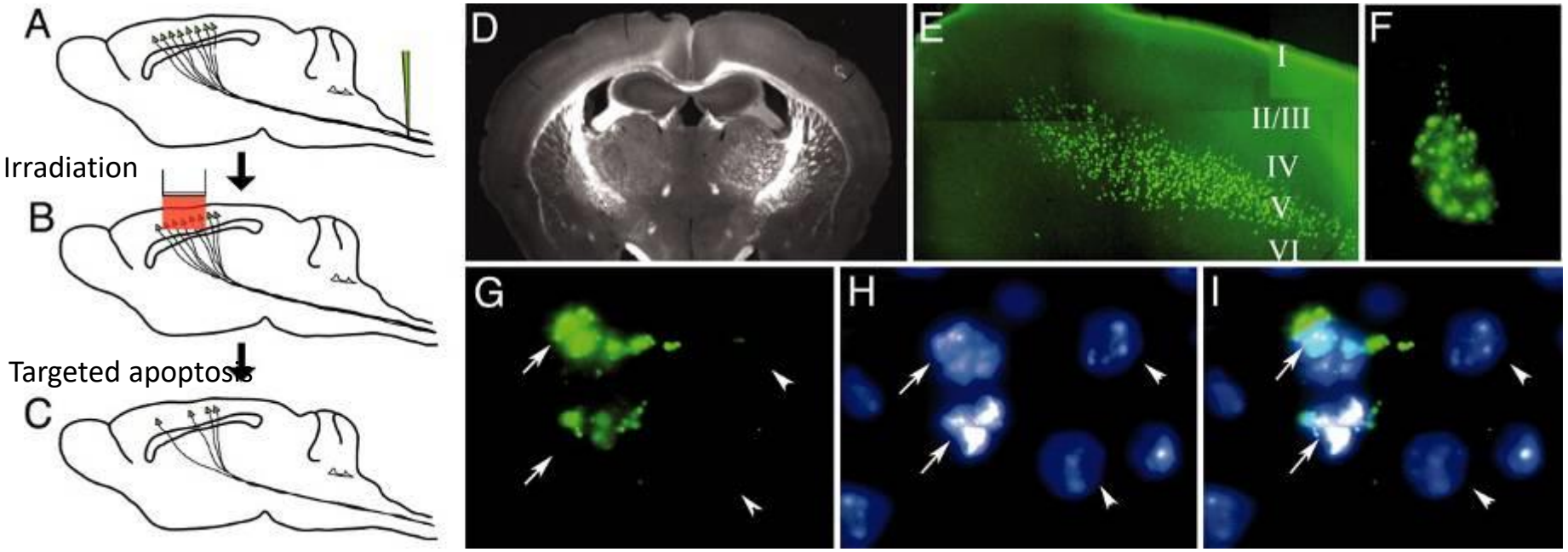
NEUROGENESIS INDUCED BY NEURODEGENERATION

Differents brain lesions can induce neurogenesis in non canonical regions

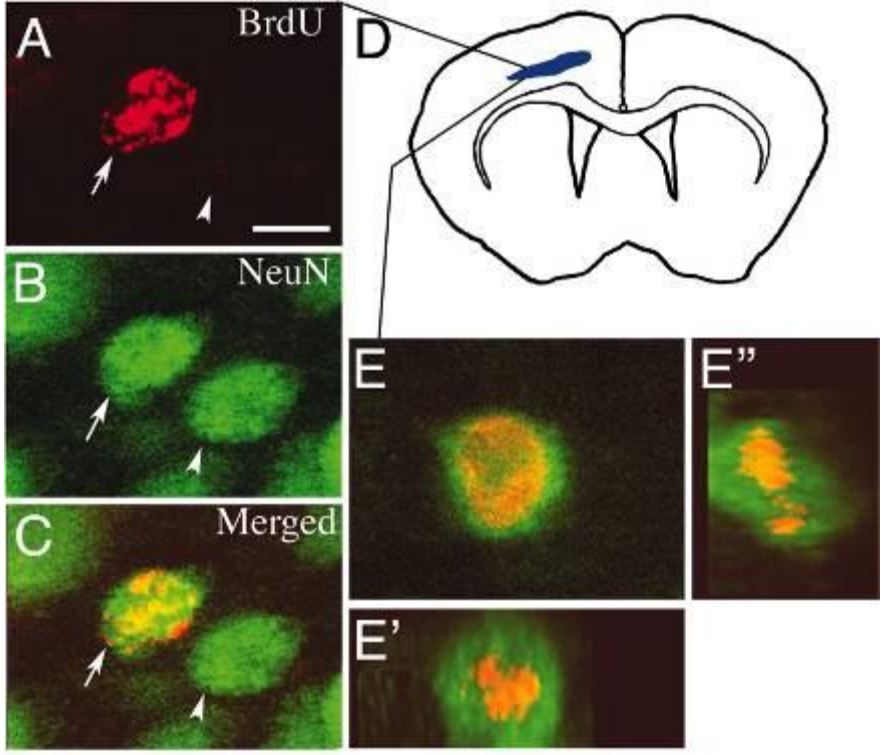
TARGETED APOPTOSIS OF CORTICO-SPINAL NEURONS.

Green fluorescent nanospheres carrying chlorin e6 were microinjected into the dorsal spinal cord. The nanospheres were retrogradely transported to the somata of layer V corticospinal motor neurons Photoactivated chlorin e6 produced singlet oxygen within neuronal lysosomes, inducing apoptosis exclusively in nanosphere-containing motor neurons.

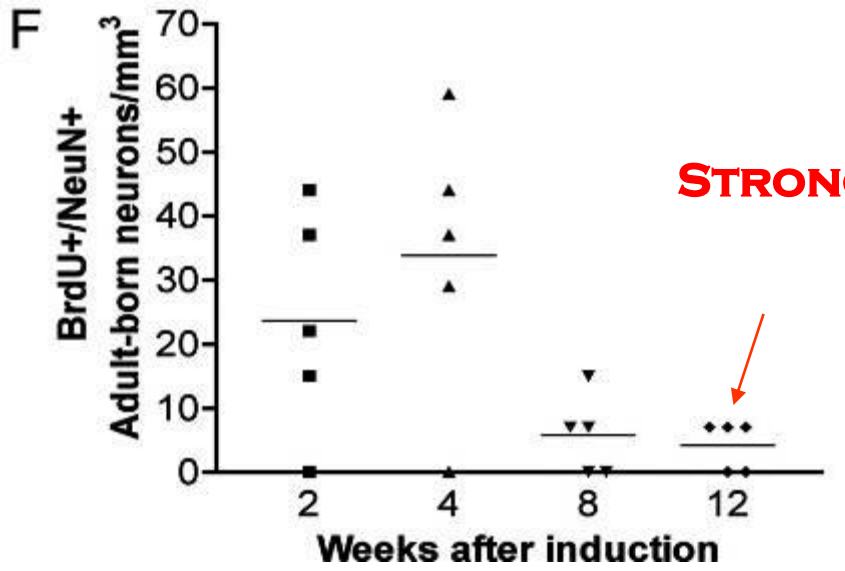
Microinjection of green fluorescent nanosphere carryng Chlorin e



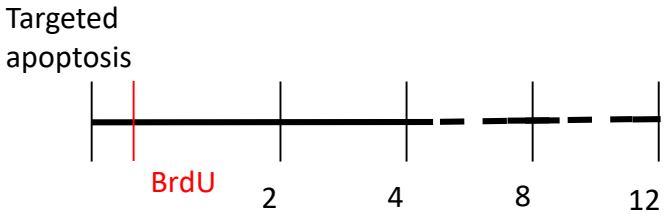
Chen J et al. PNAS 2004;101:16357-16362



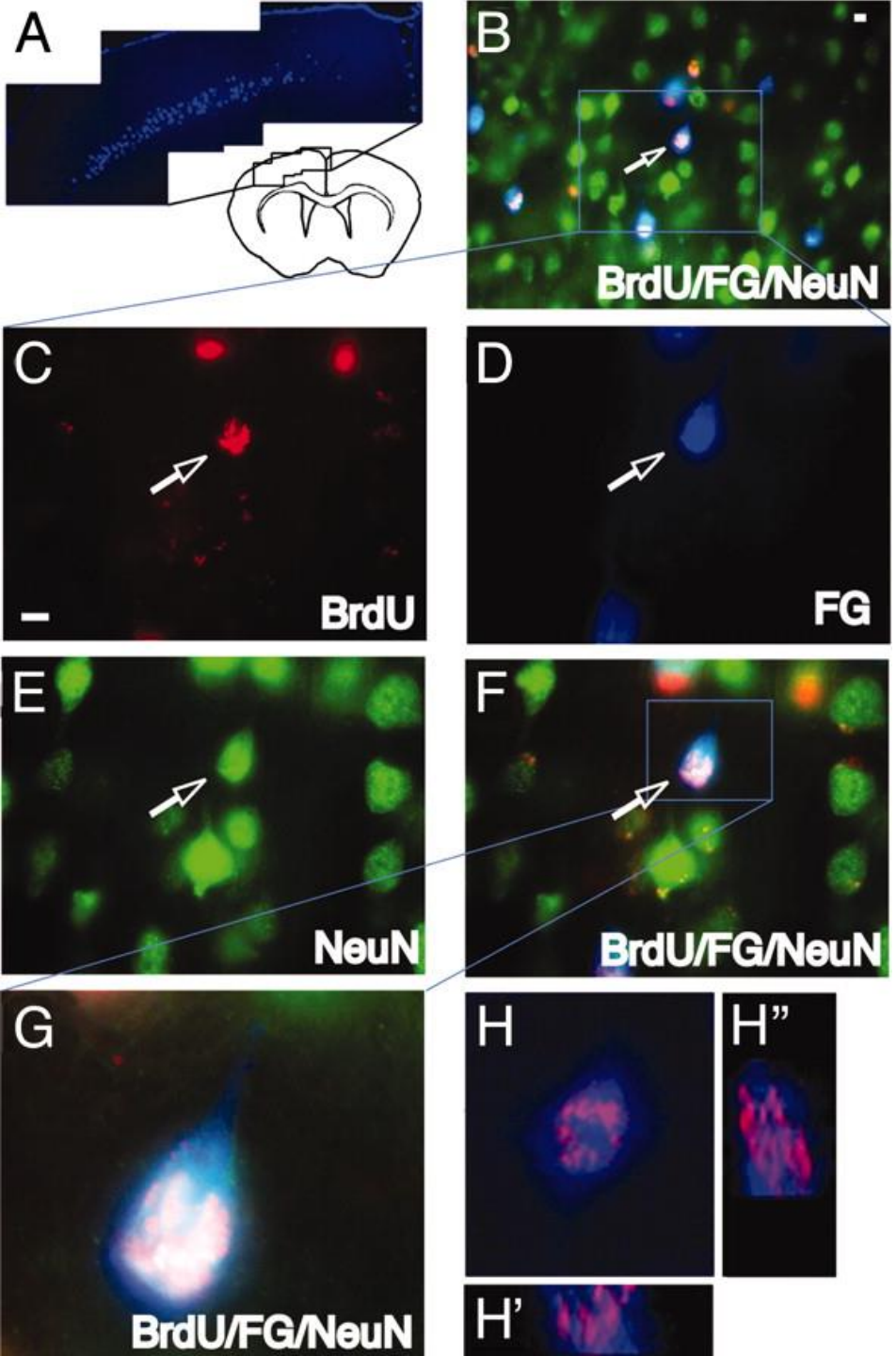
Newly generated BrdU+ cells can be induced to differentiate into mature neurons in regions of the cortex undergoing targeted apoptotic CSMN degeneration.



STRONG SELECTION!

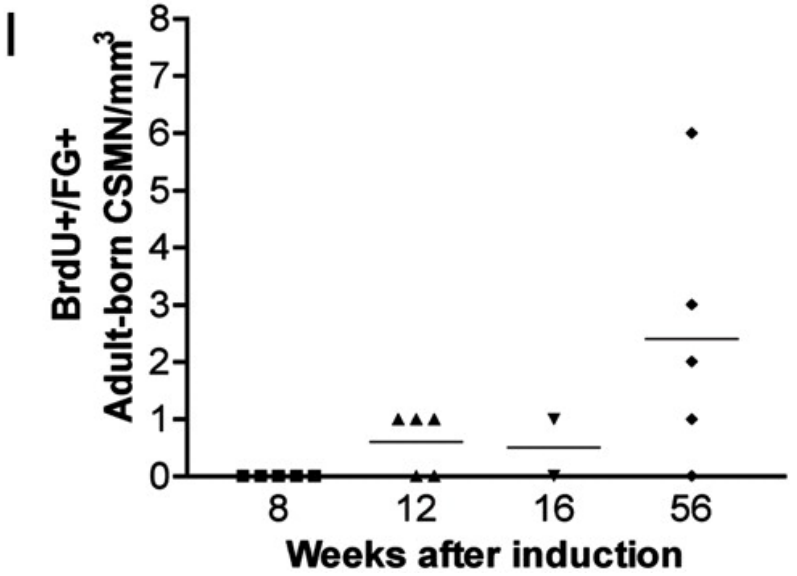


Chen J et al. PNAS 2004;101:16357-16362



A subset of newborn layer V cortical neurons extends axons to the cervical spinal cord.

They observed very few cells…



Hypothesis: ineffective attempt to replace the degenerate neurons

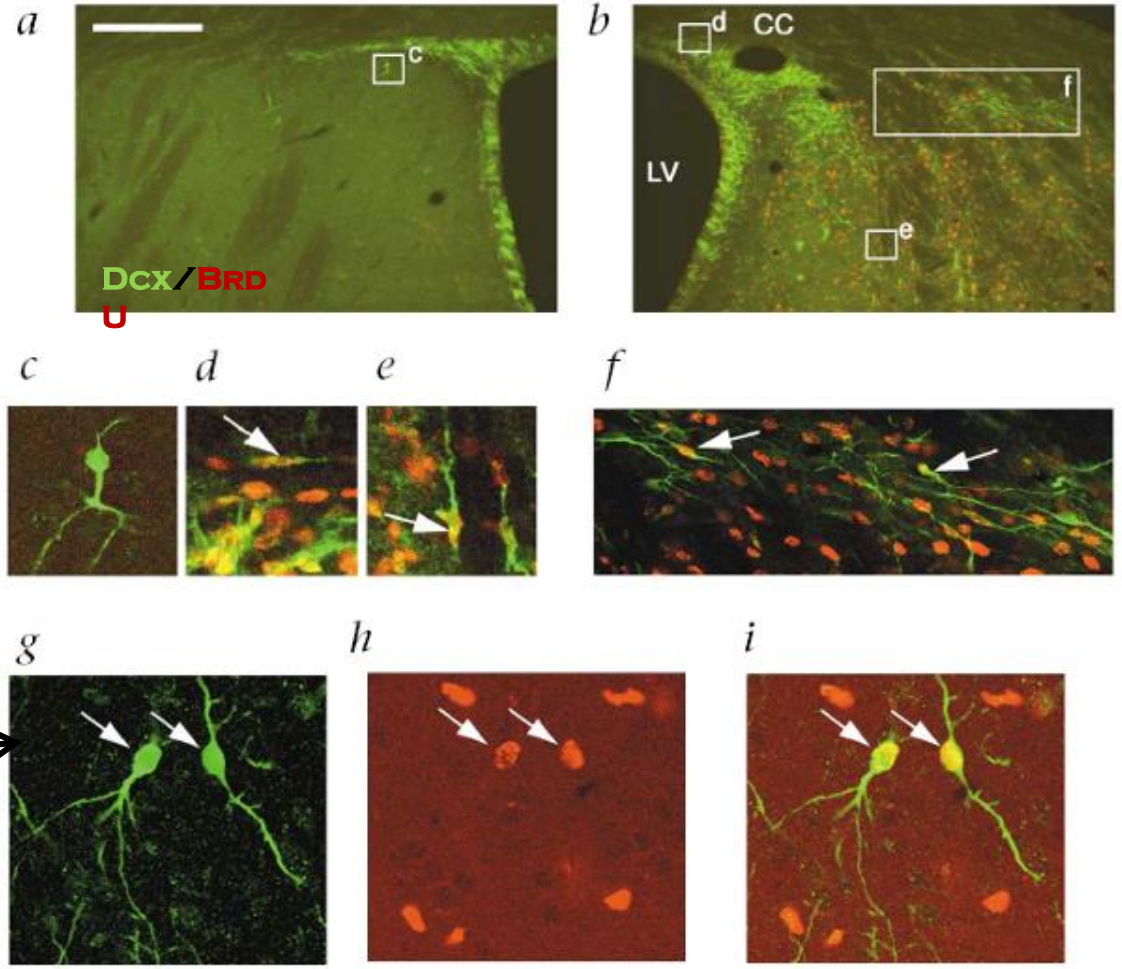
Neuronal replacement from endogenous precursors in the adult brain after stroke

ANDREAS ARVIDSSON[1], TOVE COLLIN[1], DENIZ KIRIK[2], ZAAL KOKAIA[1] & OLLE LINDVALL[1]

Nature Medicine 2002

Stroke induces increase of cell proliferation in the SVZ

Therefore, they suggested that striatal newly generated neuroblasts migrated from the SVZ to the lesioned striatum

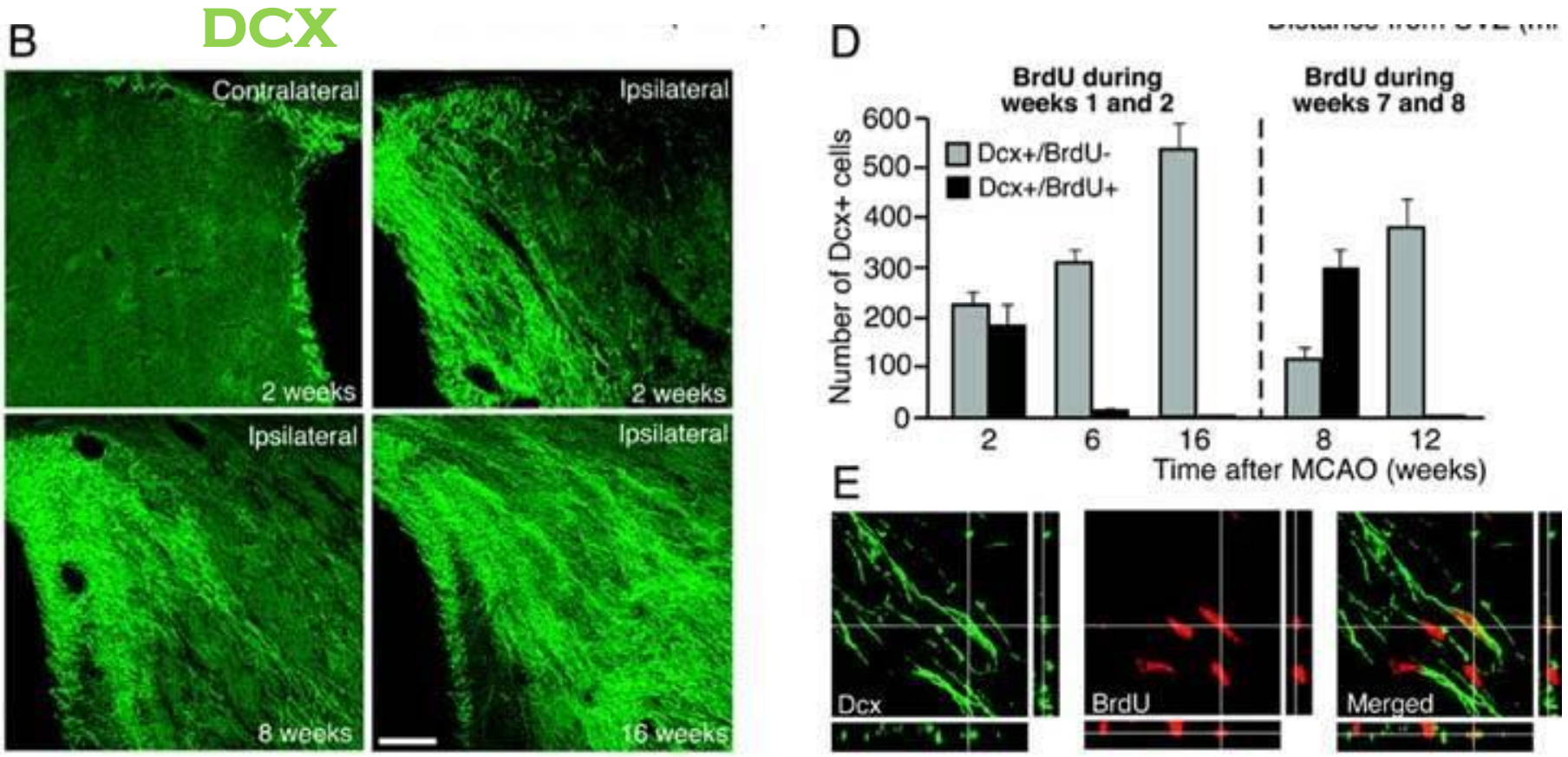


Neurons showing a mature morphology

DCX

BRDU

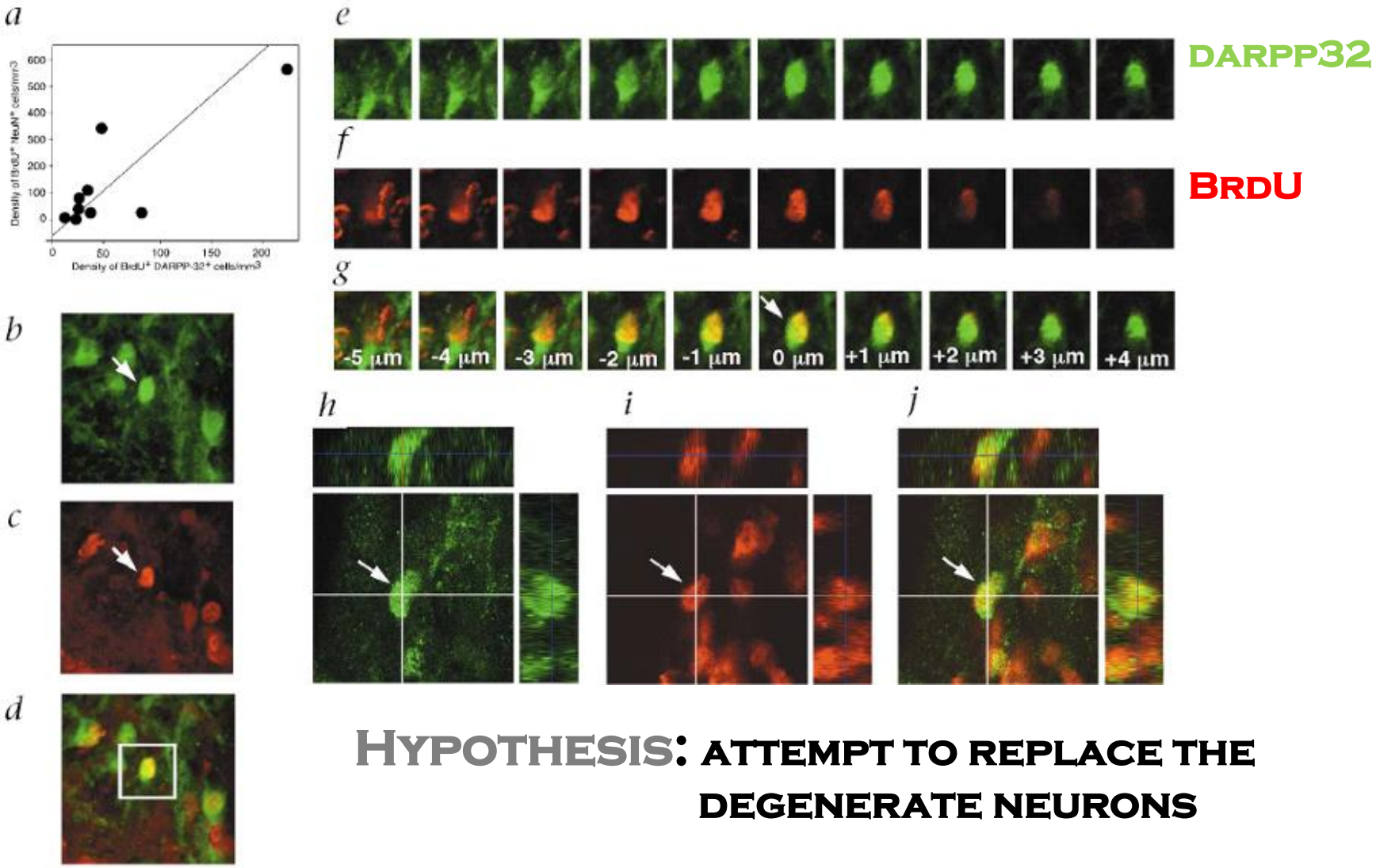
The appearance of striatal neurogenesis after stroke persists for at least four months



Thored P et al. 2006

Some cells differentiate into **DARPP32+ ,** medium spiny neurons marker

STRONG SELECTION



Hypothesis: attempt to replace the degenerate neurons

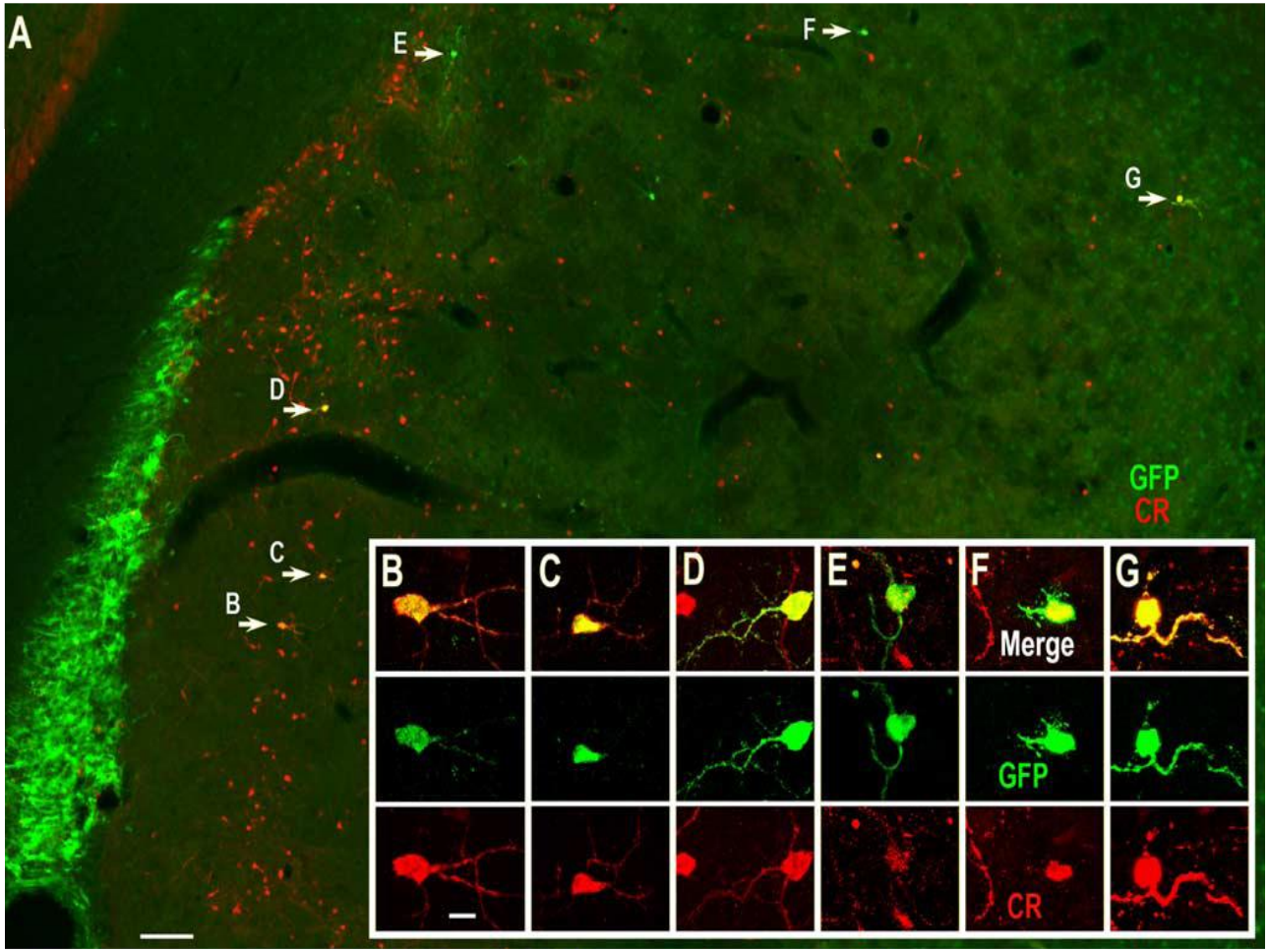
Brain Injury Does Not Alter the Intrinsic Differentiation Potential of Adult Neuroblasts

J. Neurosci. 2009

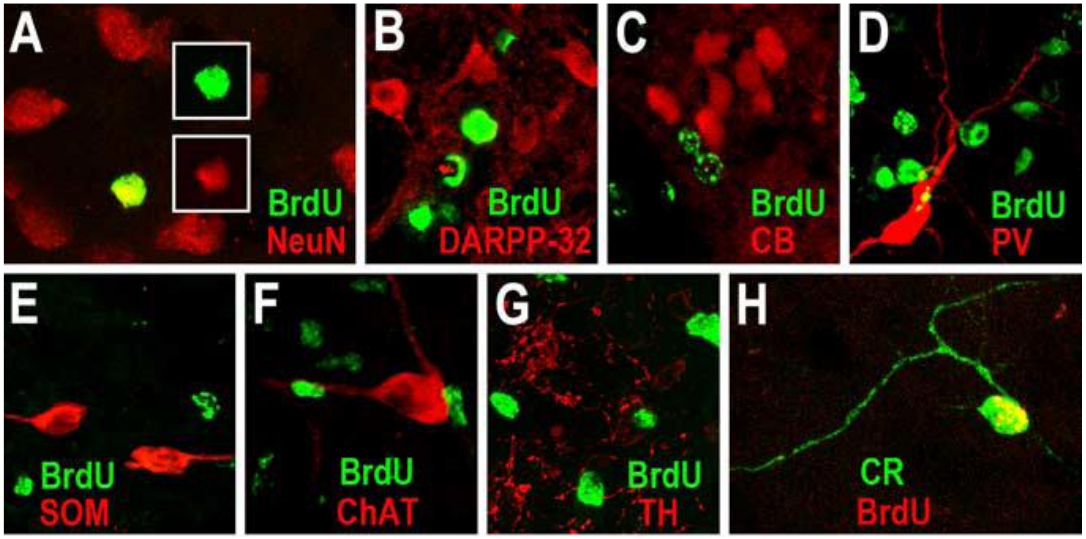
Fang Liu,[1*] Yan You,[1*] Xiaosu Li,[1] Tong Ma,[1] Yanzhen Nie,[1] Bin Wei,[1] Tiejun Li,[2] Huanbing Lin,[3] and Zhengang Yang[1]

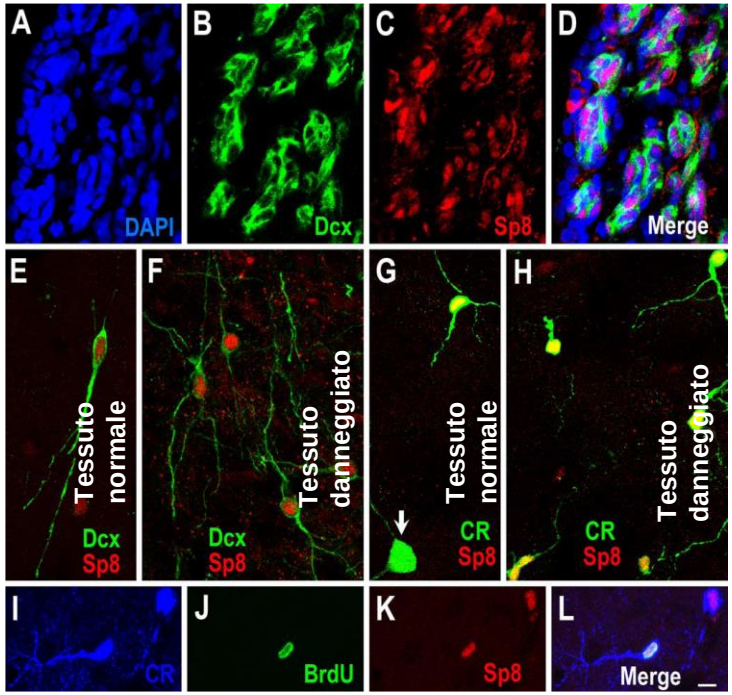
Lesion: Stroke

Through stereotaxic injections of a retroviral vector encoding the GFP reporter gene in the rat SVZ, Liu et al., 2009 demonstrated the SVZ origin of at least part of the newly generated striatal neurons after MCAO.



Newborn neurons do not express markers of mature striatal neurons nor transcription factors involved in their specification (for example Ctip2, Foxp1)





A few cells, less than 5% of the initial population of newly generated neurons, express calretinin, a marker of a small subtype of striatal, but also OB interneurons

Newborn neurons in the striatum express Sp8, a transcription factor associated to the olfactory bulb interneurons specification.

Strong selection

Hypothesis

Considering that:

1. Sp8 is associated with the specification of OB interneurons,

2. calretinin expression is restricted to a very small population of striatal neurons and is also expressed by OB interneurons

IT HAS BEEN PROPOSED THAT NEWLY BORN NEURONS IN THE DAMAGED STRIATUM COULD REPRESENT MISDIRECTED OB NEURONS

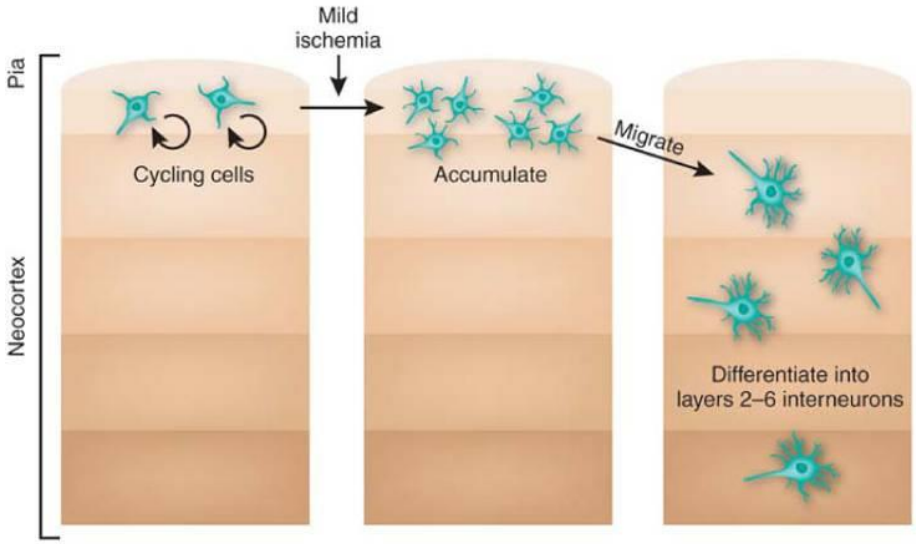
Ischemia-induced neurogenesis of neocortical layer 1 progenitor cells

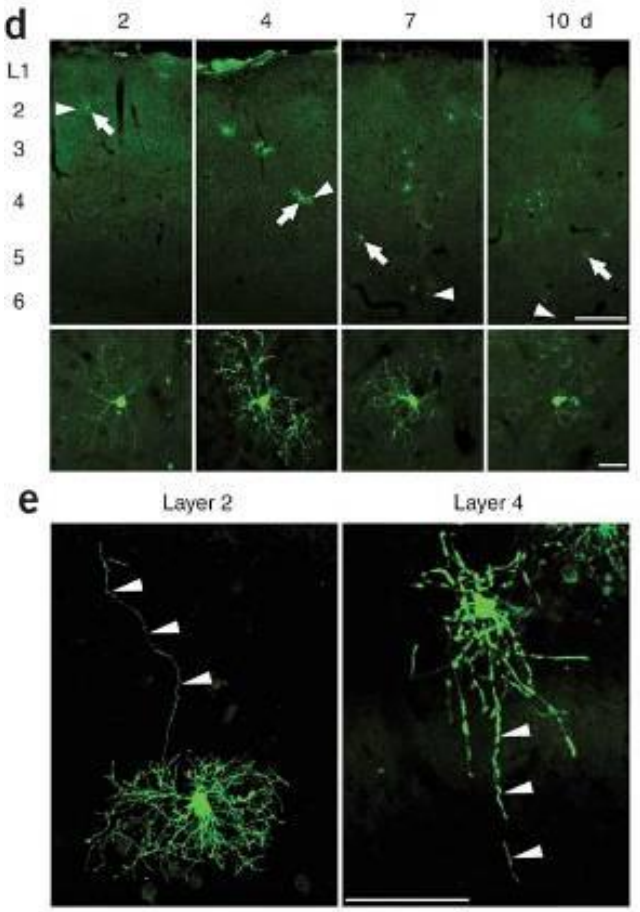
Koji Ohira[1–4], Takahiro Furuta[2], Hiroyuki Hioki[2], Kouichi C Nakamura[2,4,8], Eriko Kuramoto[2], Yasuyo Tanaka[2], Nobuo Funatsu[3], Keiko Shimizu[5], Takao Oishi[5], Motoharu Hayashi[5], Tsuyoshi Miyakawa[1,4,6], Takeshi Kaneko[2,4] & Shun Nakamura[3,4,7]

Nature Neuroscience 2010

Mild ishemia: carotid arteries of rats were occluded for 10 min.

→ increase in the number of microglia cells
→ did not induction of cell death of NeuN+ cells
→ increase in the number of Ki67-positive cells





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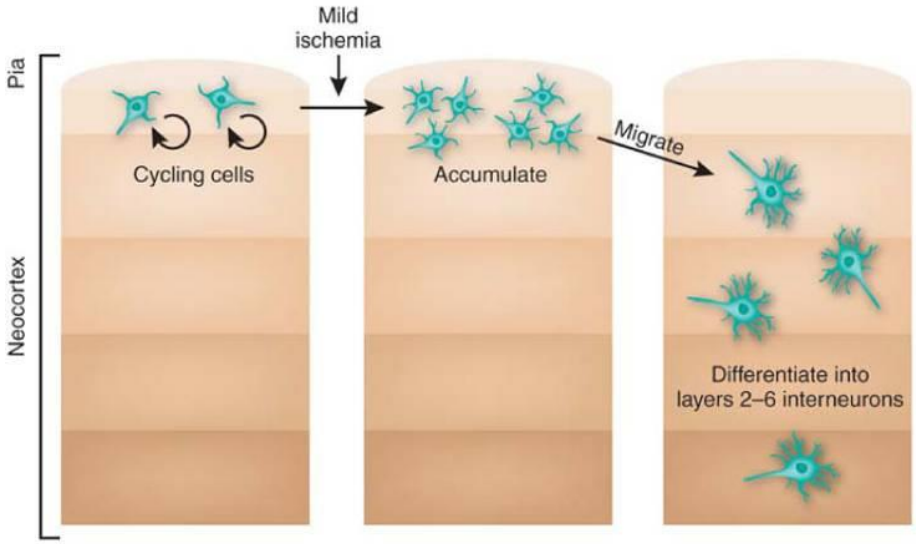
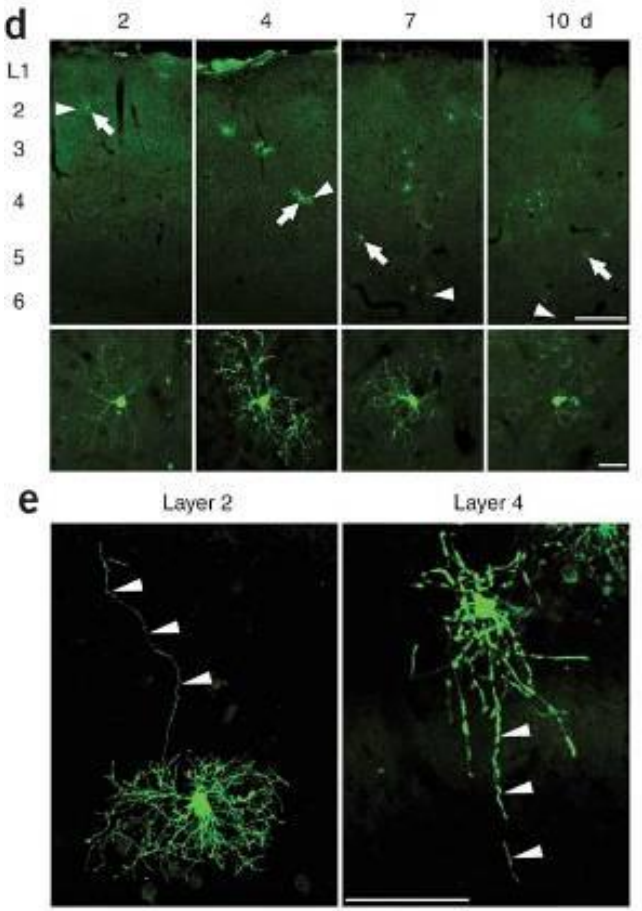
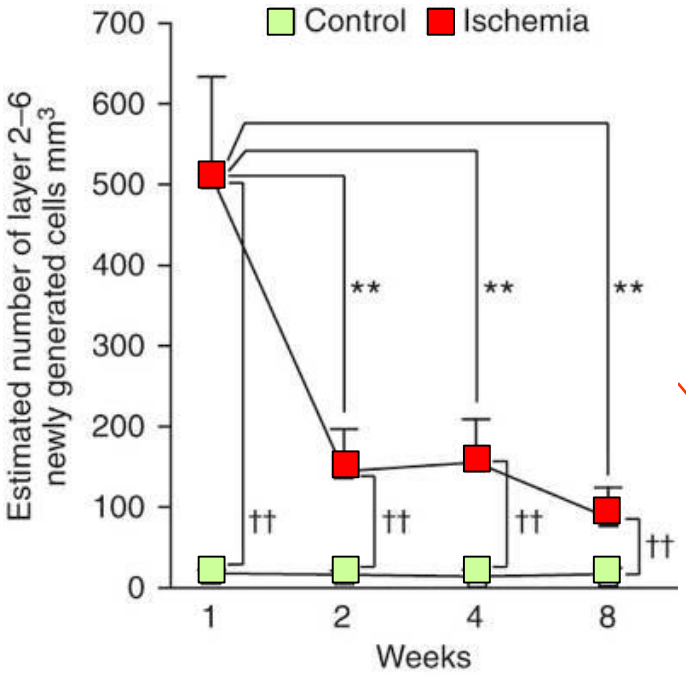




They injected a retrovirus expressing the GFP reporter into the layer 1 of the neocortex 1 day after ischemia

Newborn neurons generated after lesion migrated to the deepest layers

In contrast, few neurons were labelled when the retrovirus was injected into the SVZ



Local progenitors and transient neurons.

Newbor neurons differentiated into GABAergic interneurons, they expressed neuropeptide Y (NPY), somatostatin (SOM)

STRONG SELECTION!

Hypothesis

anti-convulsant and
anti-epileptogenic functions

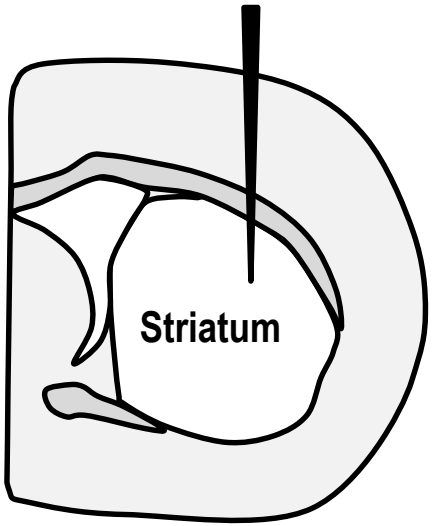
MODEL OF ACUTE NEURODEGENERATION
INTRASTRIATAL INJECTION OF QUINOLINIC ACID (QA)

RESEARCH REPORT STEM CELLS AND REGENERATION

Striatal astrocytes produce neuroblasts in an excitotoxic model of Huntington's disease

Giulia Nato[1,2], Alessia Caramello[1,2], Sara Trova[1,2], Valeria Avataneo[1,2], Chiara Rolando[3], Verdon Taylor[3], Annalisa Buffo[2,4], Paolo Peretto[1,2,*,‡] and Federico Luzzati[1,2,*,‡]

DEVELOPMENT



NMDAr agonist that causes excitotoxic death

↓

Selective loss of GABAergic

medium spiny projection neurons

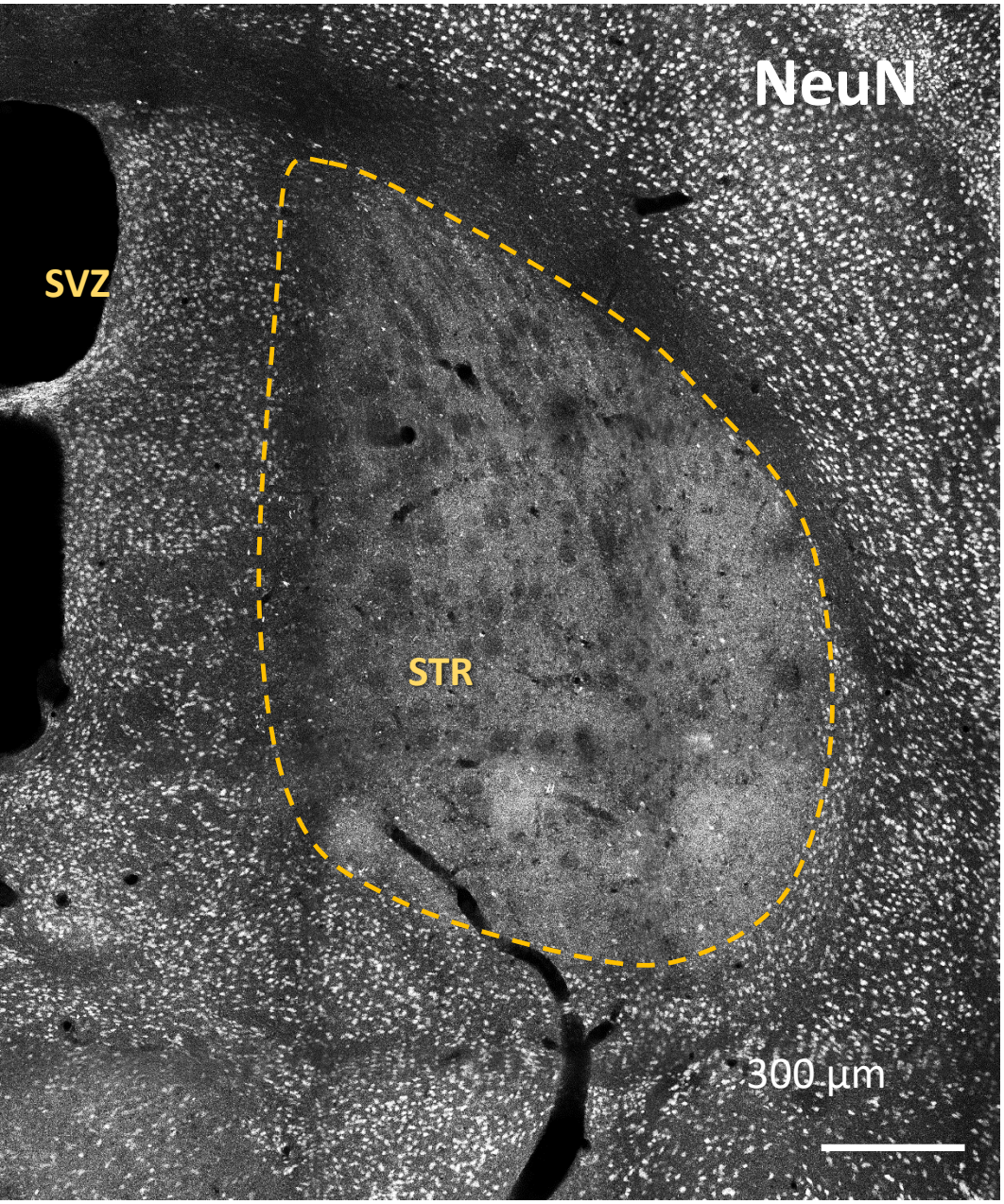
(95% of all striatal neurons)

↓

motor alterations in lesioned animals

The QA injection into the rodent brain reproduces a neurodegenerative phenotype resembling that observed in patients suffering from Huntington's disease.

QA INDUCES NEURONAL DEGENERATION

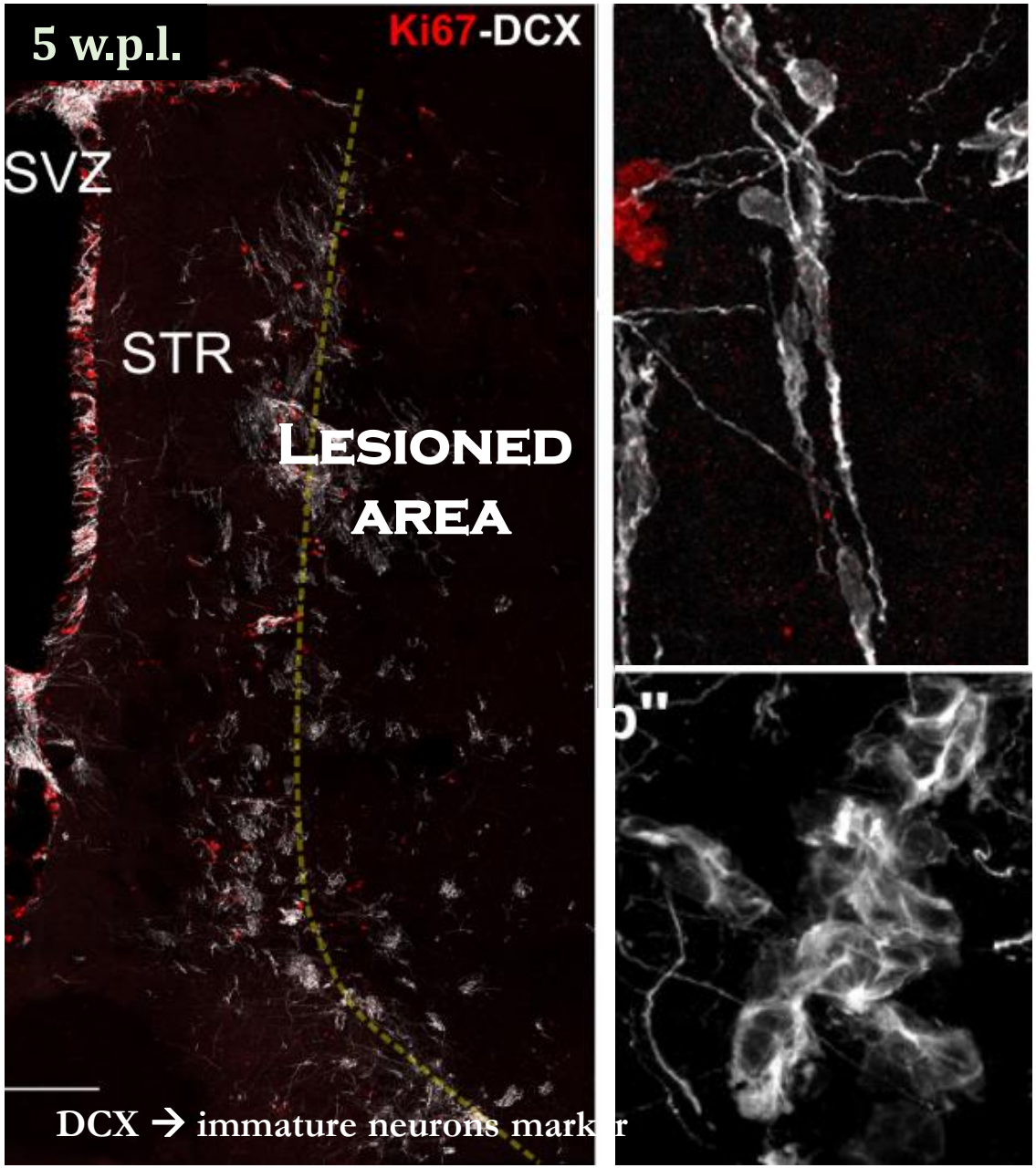


Strong loss of neurons

NeuN → mature neurons marker

5 weeks post lesion

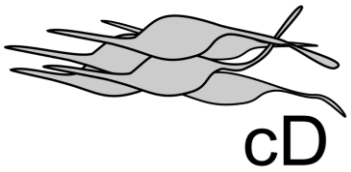
QA LESION INDUCES A NEUROGENIC RESPONSE



DCX+ neuroblasts organised into:

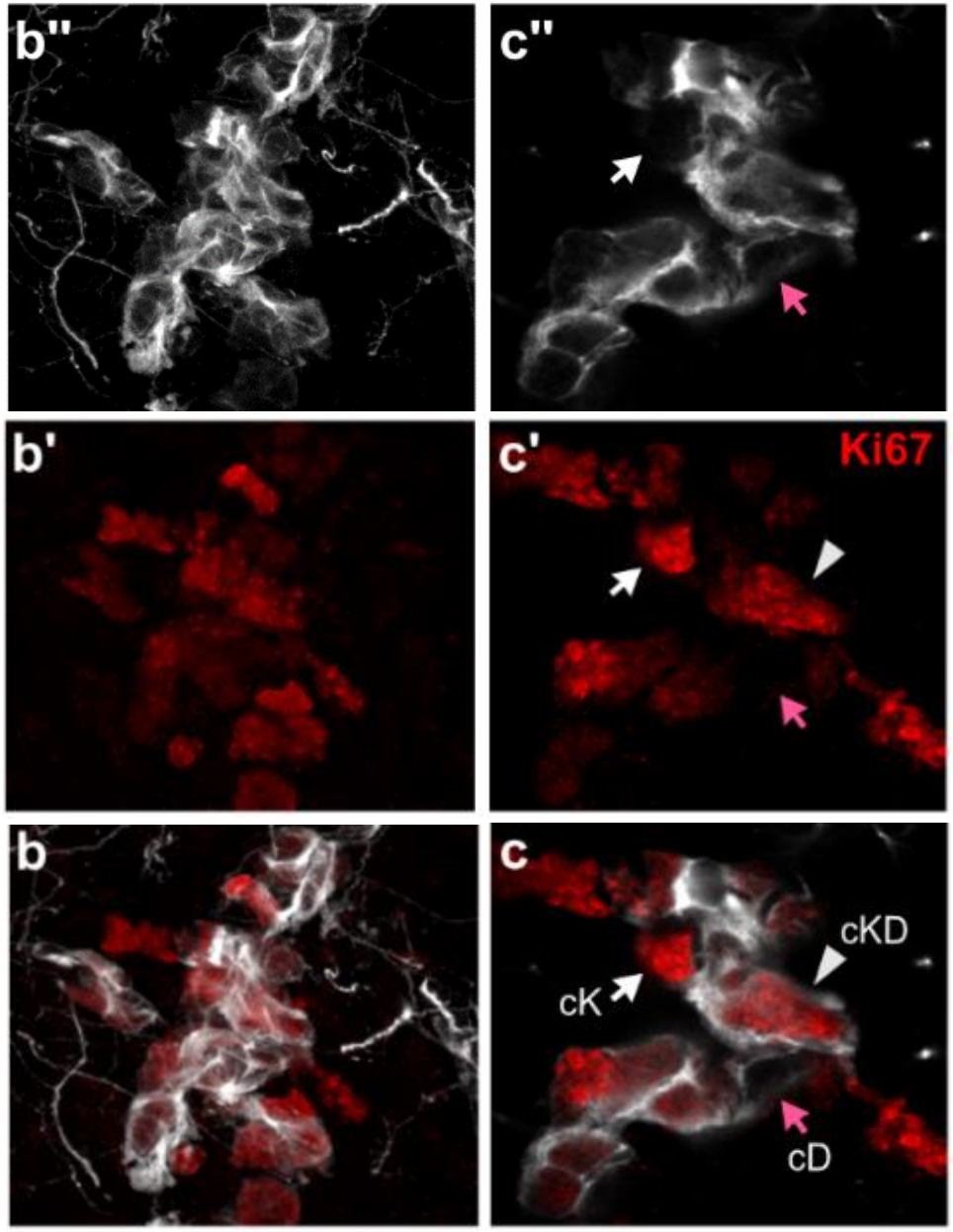
- individual cells (iD)

- clusters (cD)

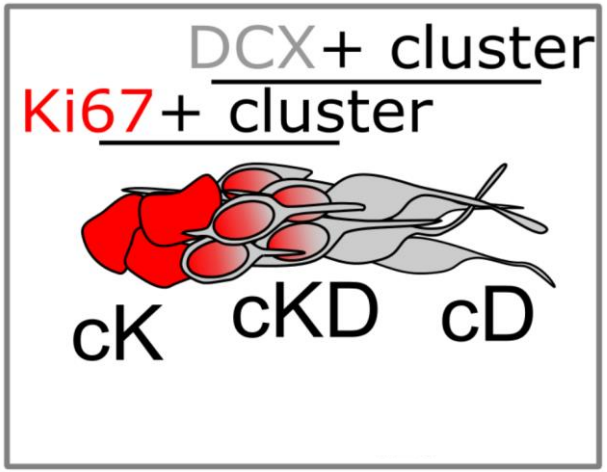


QA LESION INDUCES A NEUROGENIC RESPONSE

Max intensity projection Single optical plane



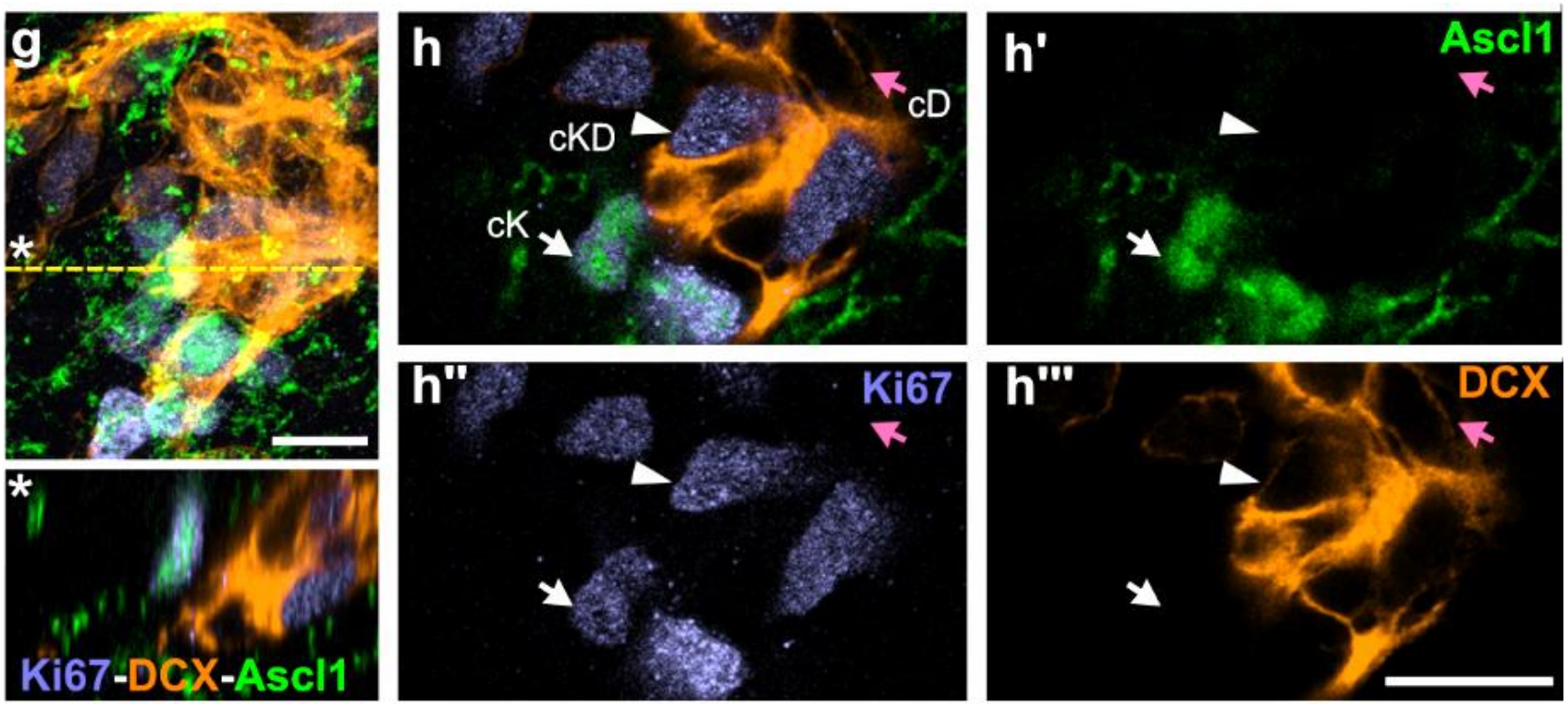
The clusters of DCX+ cells were closely associated to clusters of cells expressing the proliferation marker Ki67



Ki67
→ PROLIFERATING CELL MARKER

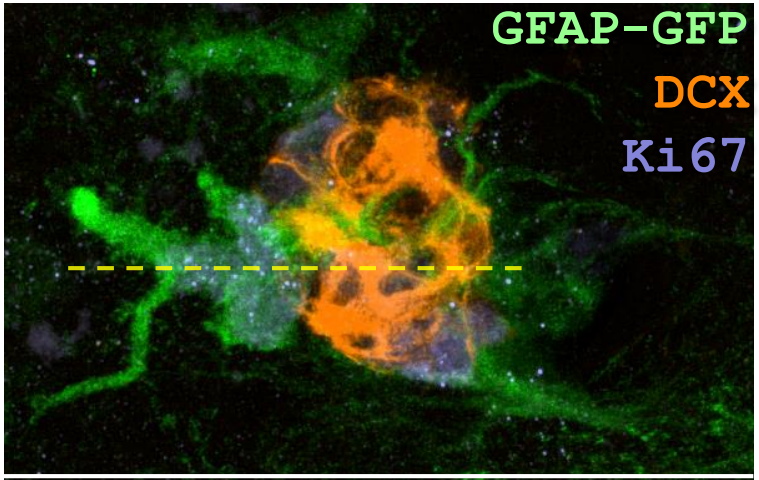
QA STIMULATES THE APPEARANCE OF TAP-LIKE PROGENITORS (ki67+ cells in clusters, cK cells)

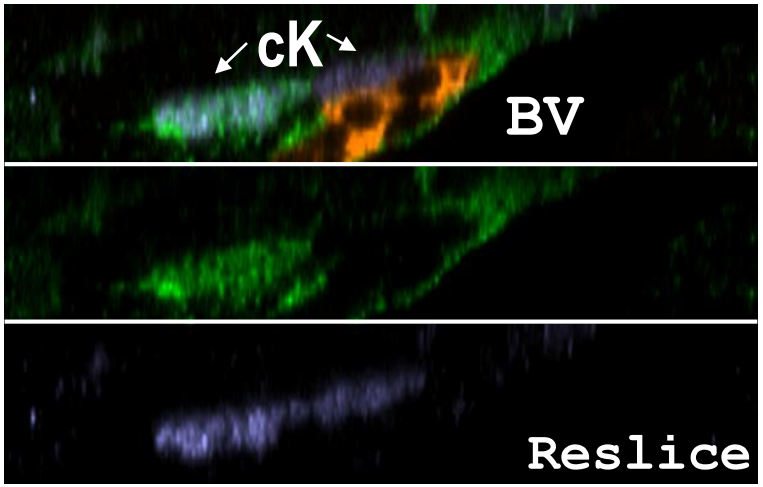
that give rise to neuroblasts that initially cluster (cKD,cDcells) and subsequently disperse as individual cells (iD cells)

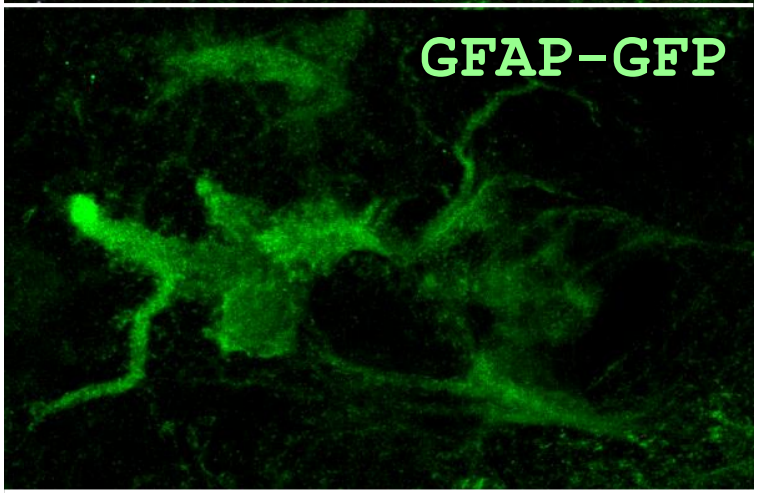


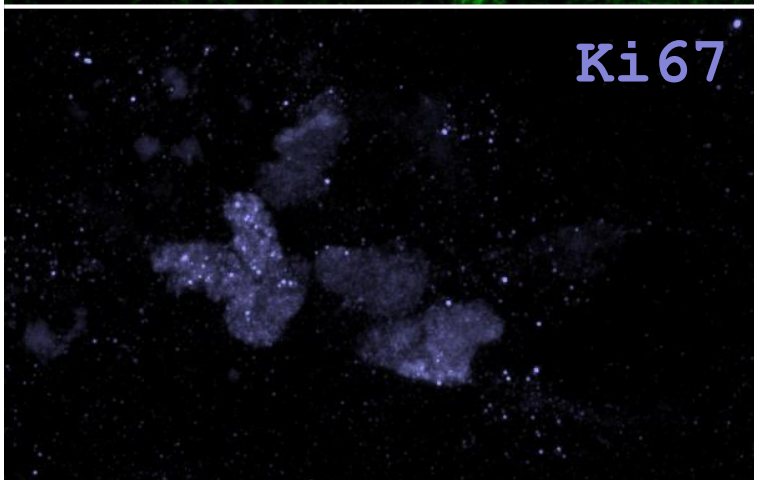
Ki67+cells in clusters expressed the TAP markers as **ASCL1**

DCX → IMMATURE NEURONS MARKER
Ki67 → PROLIFERATING CELLMARKER





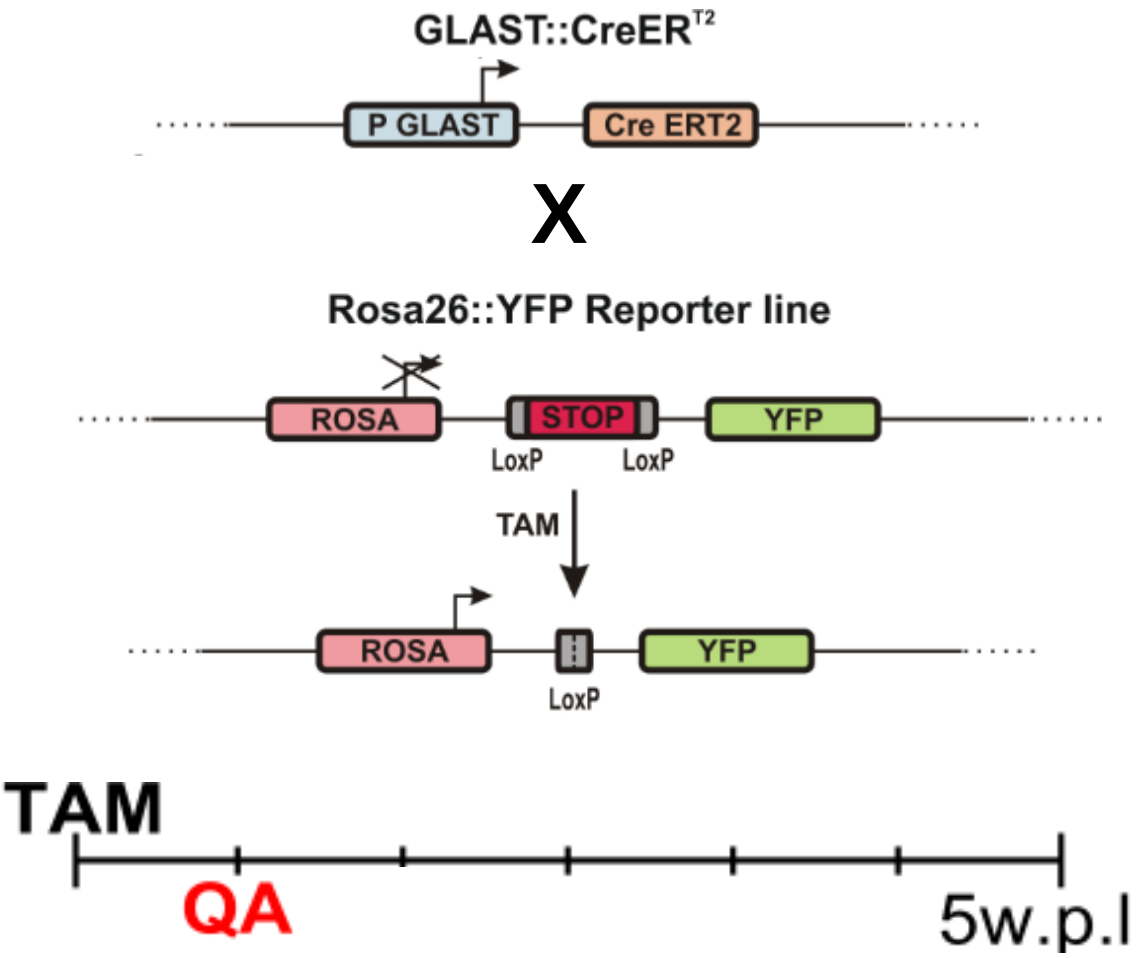


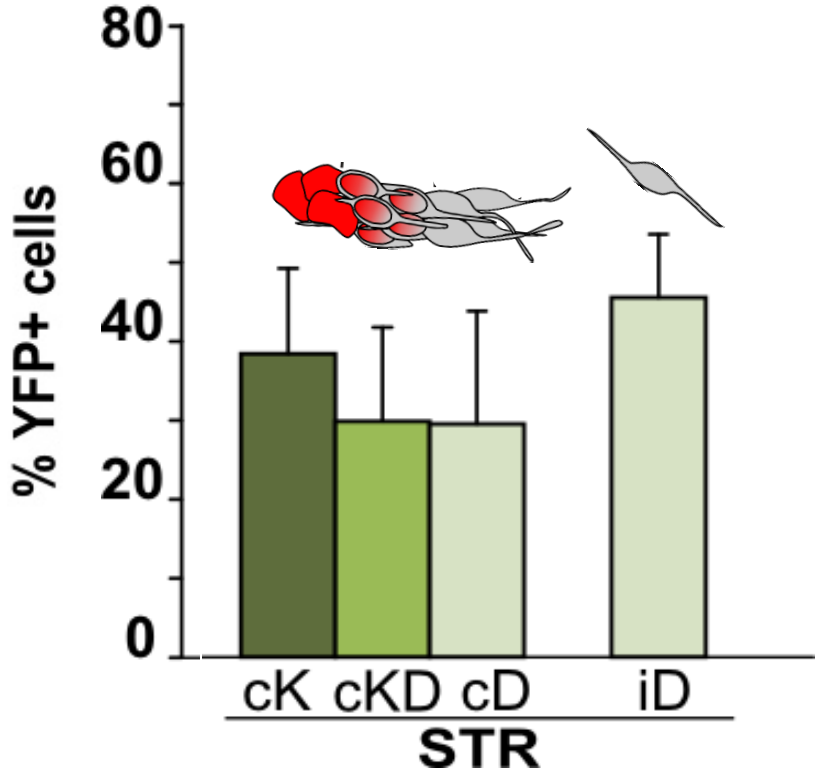


The 8% of Ki67+ cells in clusters express GFP in GFAP-GFP mice

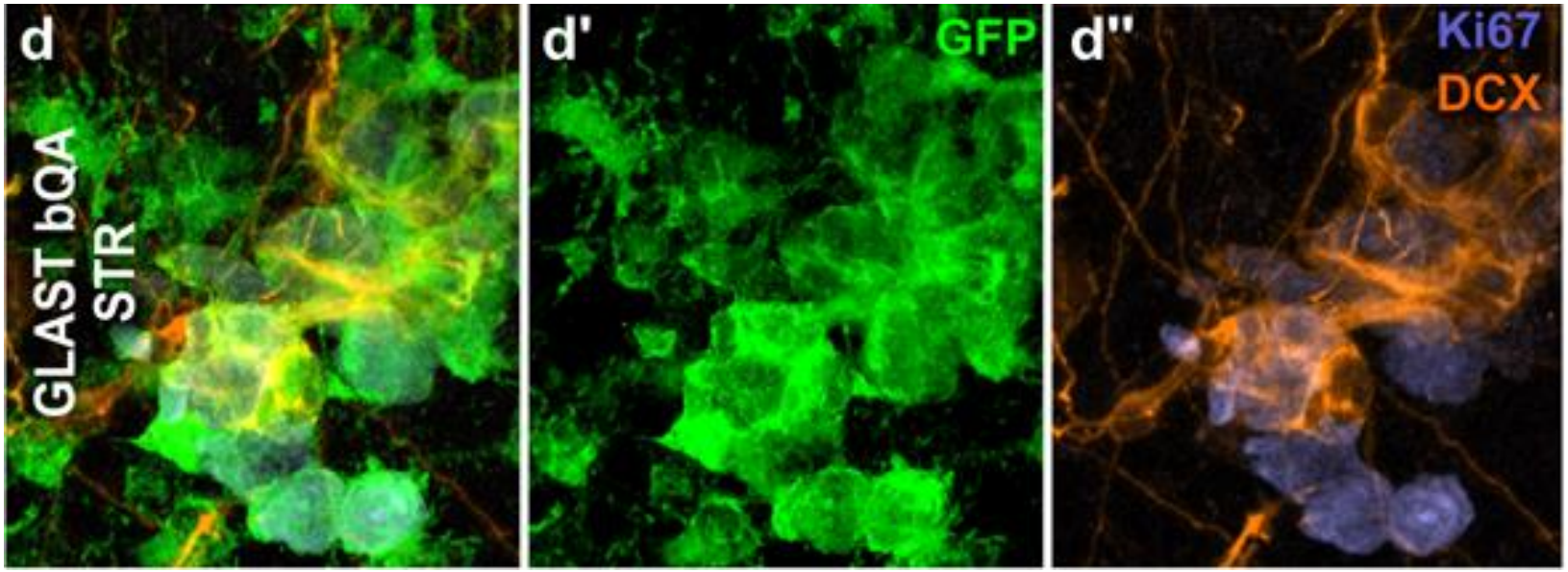
GFAP → expressed by astrocytes

INDUCIBLE GENETIC FATE MAPPING
GLAST: PAN ASTROCYTIC MARKER





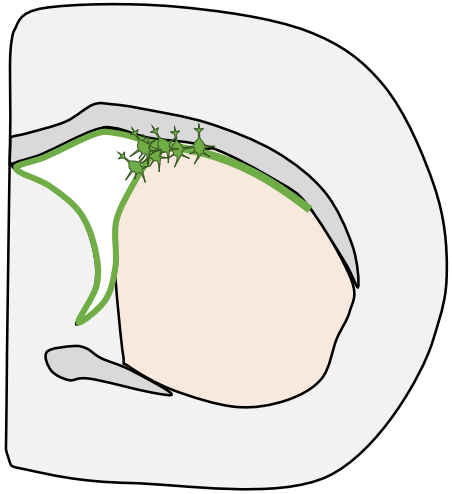
GLAST+ ASTROCYTES GENERATE Ki76+ CLUSTERS AND DCX+ NEUROBLASTS



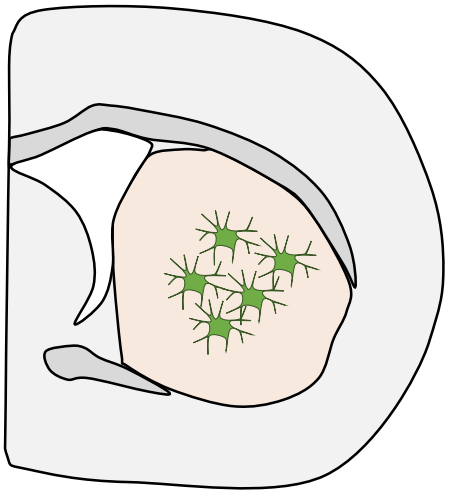
ORIGIN OF THE NEUROGENIC PROGENITORS

Understand whether striatal TAPs and neuroblasts

1. migrate from **already active neurogenic niches**



2. are generated from **local astrocytes**



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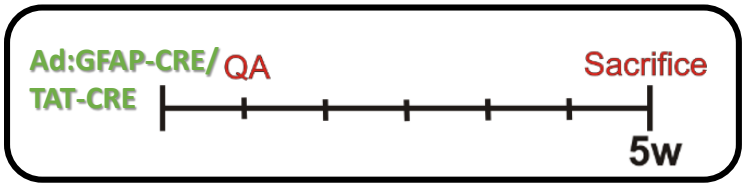
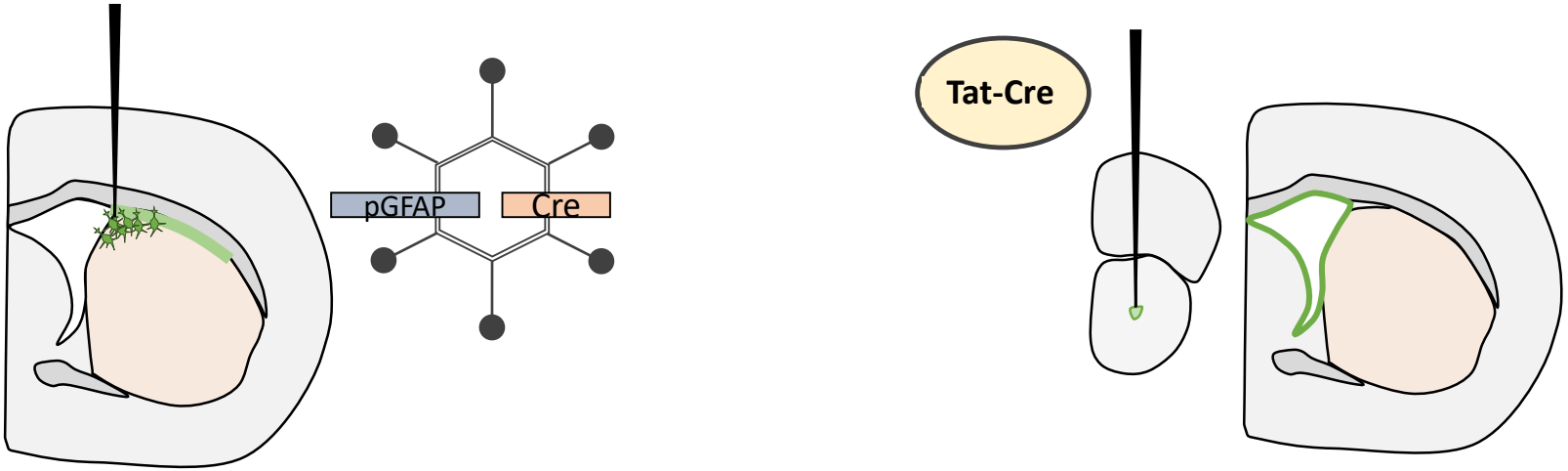
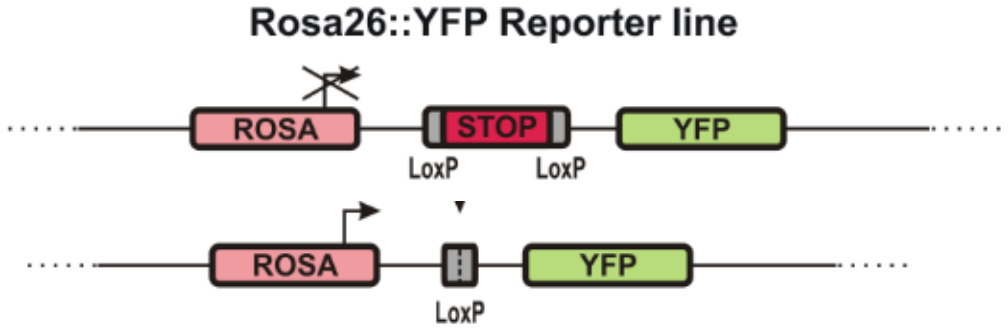
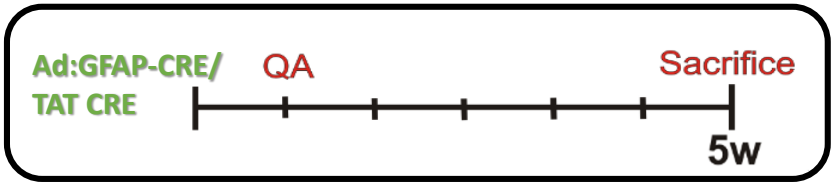
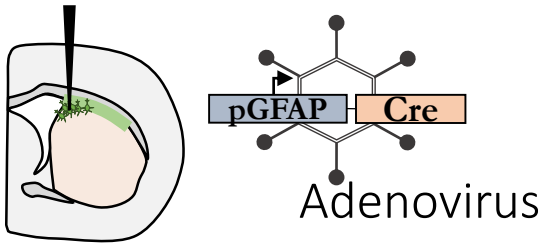


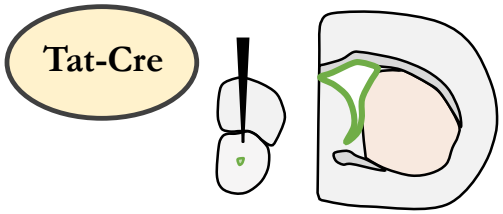


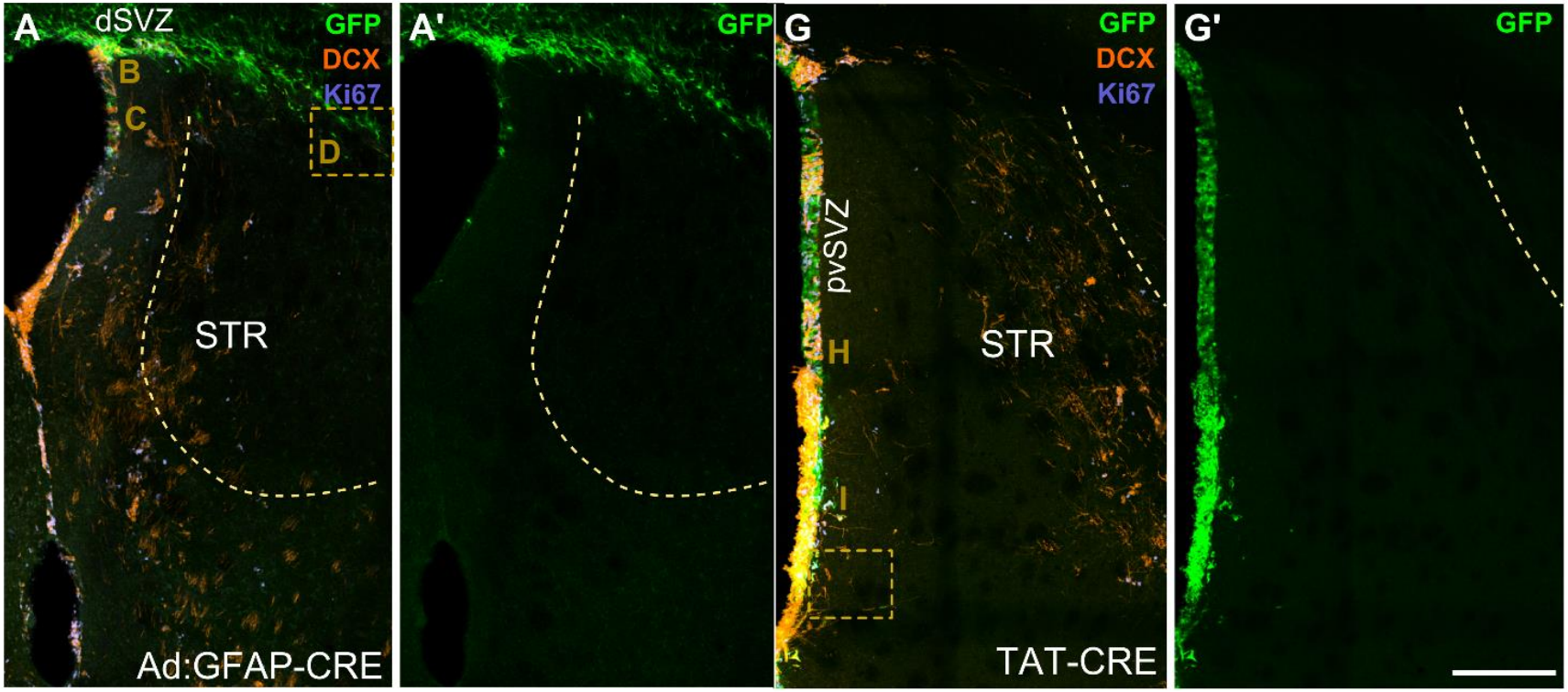


SVZ PROGENITORS DO NOT CONTRIBUTE TO THE INTRASTRIATAL TAPS



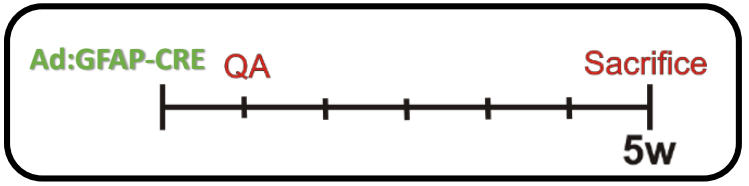


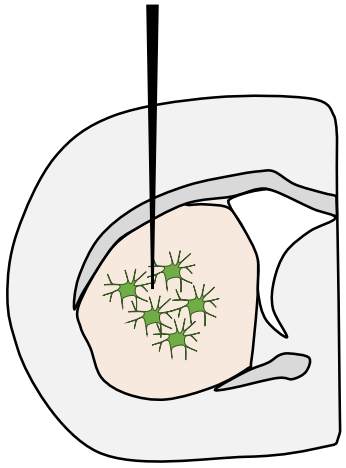


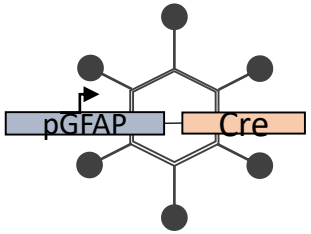


Only a few striatal iD cells express YFP

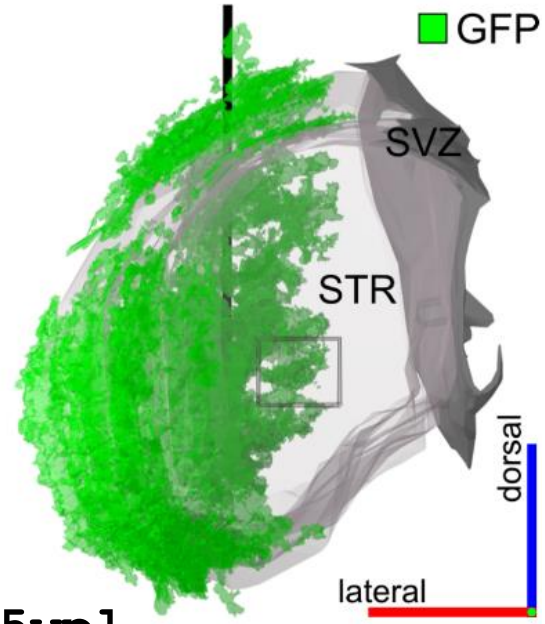
ARE STRIATAL TAPs AND NEUROBLASTS GENERATED FROM STRIATAL ASTROCYTES?





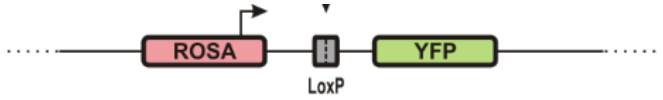


Adenovirus



5wp1

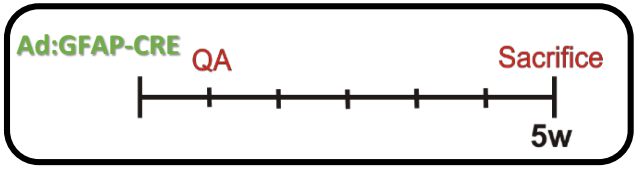
Rosa26::YFP Reporter line



Intrastriatal injection of Ad:GFAP-Cre

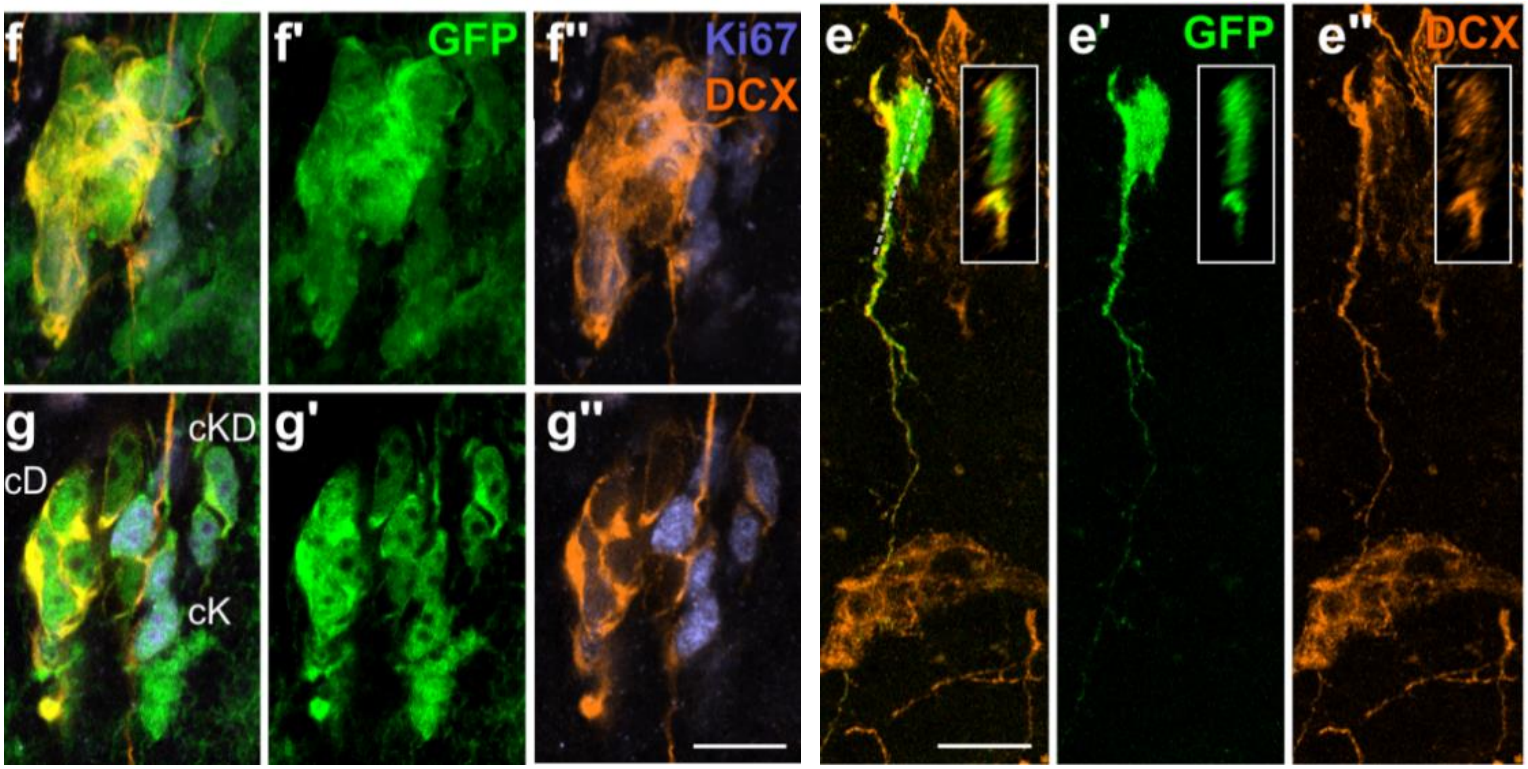
Injection of adenoviral vector carrying Cre recombinase under the control of the mouse Gfap promoter In ROSA YFP mice

STRIATAL CELLS GENERATE TAPS AND NEUROBLASTS AFTER LESION



DCX and Ki67 Clusters

Individual neuroblasts

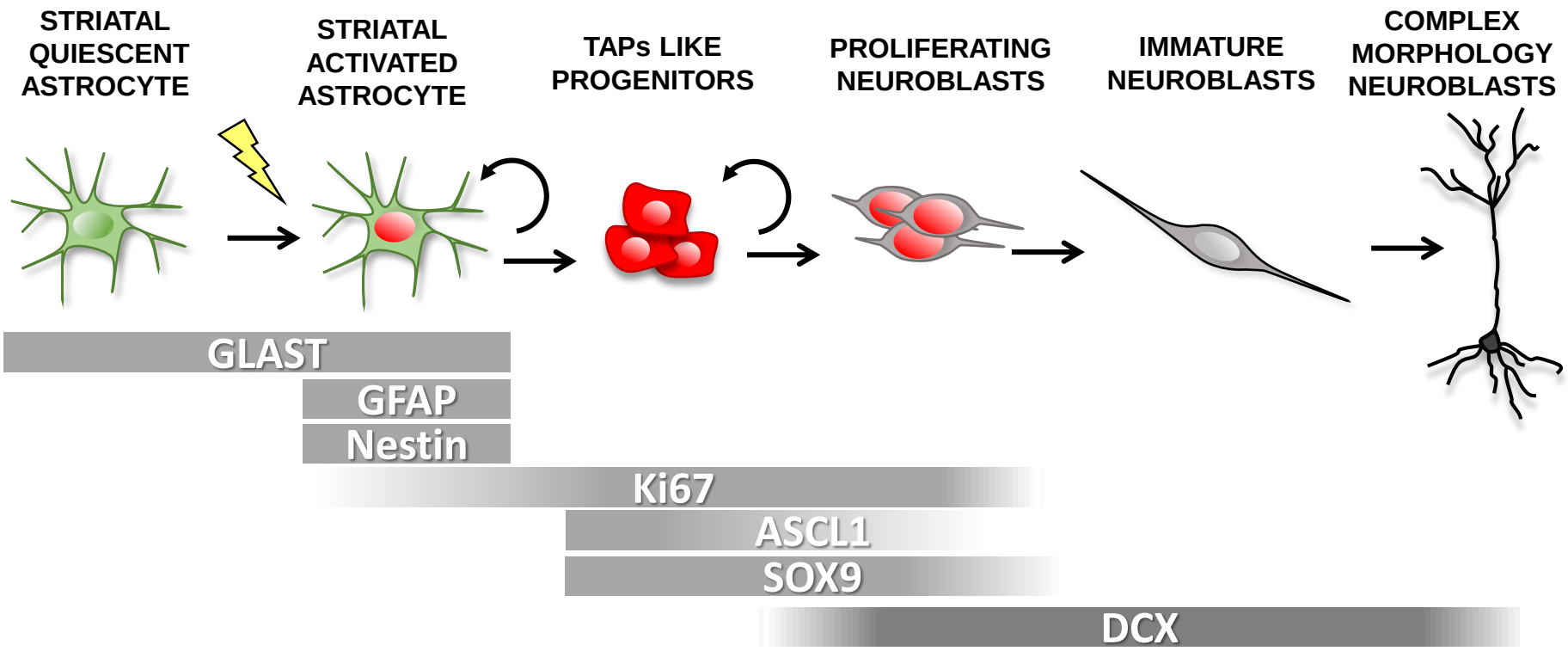


The Ad:GFAP-Cre injection demonstrates the striatal origin of the neurogenic progenitors and confirms their astrocytic identity

MODEL OF STRIATAL NEUROGENESIS INDUCED AFTER QA INJECTION

Striatal astrocytes are **quiescent neuronal progenitors** that become **activated after QA lesion**.

These cells produce neurons through **TAPs like cells**

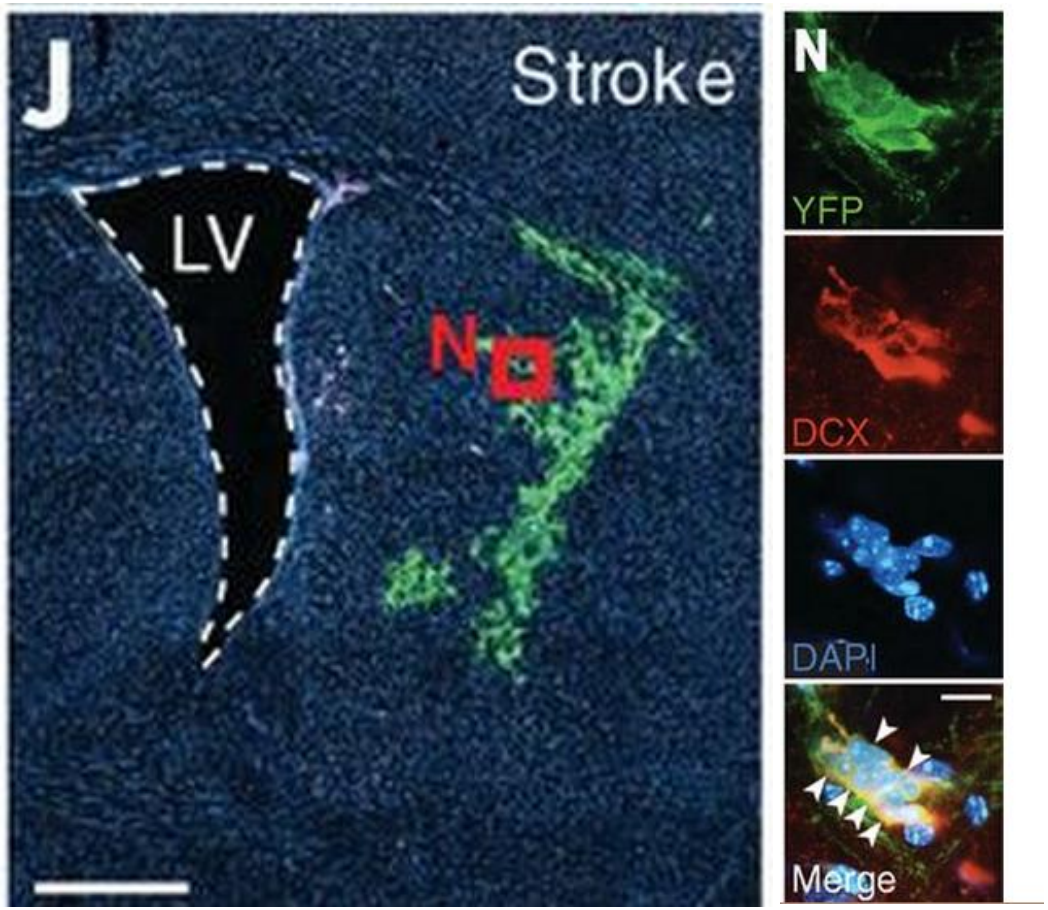


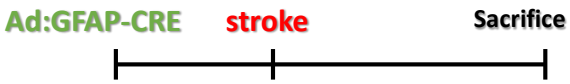
Nato et al., 2015. Development

striatal astrocytes
generated neuroblasts after the stroke

A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse

Jens P. Magnusson,[1*] Christian Göritz,[1*] Jemal Tatarishvili,[2] David O. Dias,[1] Emma M. K. Smith,[3] Olle Lindvall,[2] Zaal Kokaia,[2] Jonas Frisén[1†]





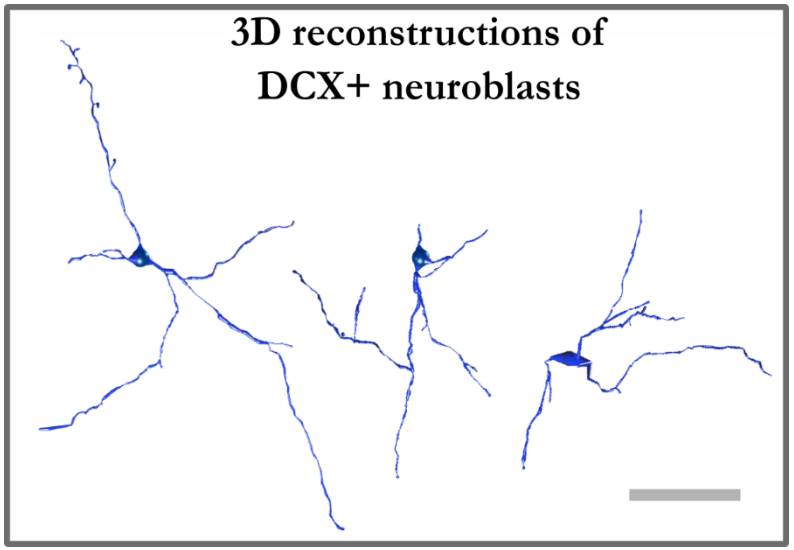
IDENTITY OF NEWBORN STRIATAL NEUROBLASTS

- **Do not express markers of known classes of striatal neurons**(very few express calretinin and nNOS)
- **Express Sp8** (LGE/CGE derived interneurons)

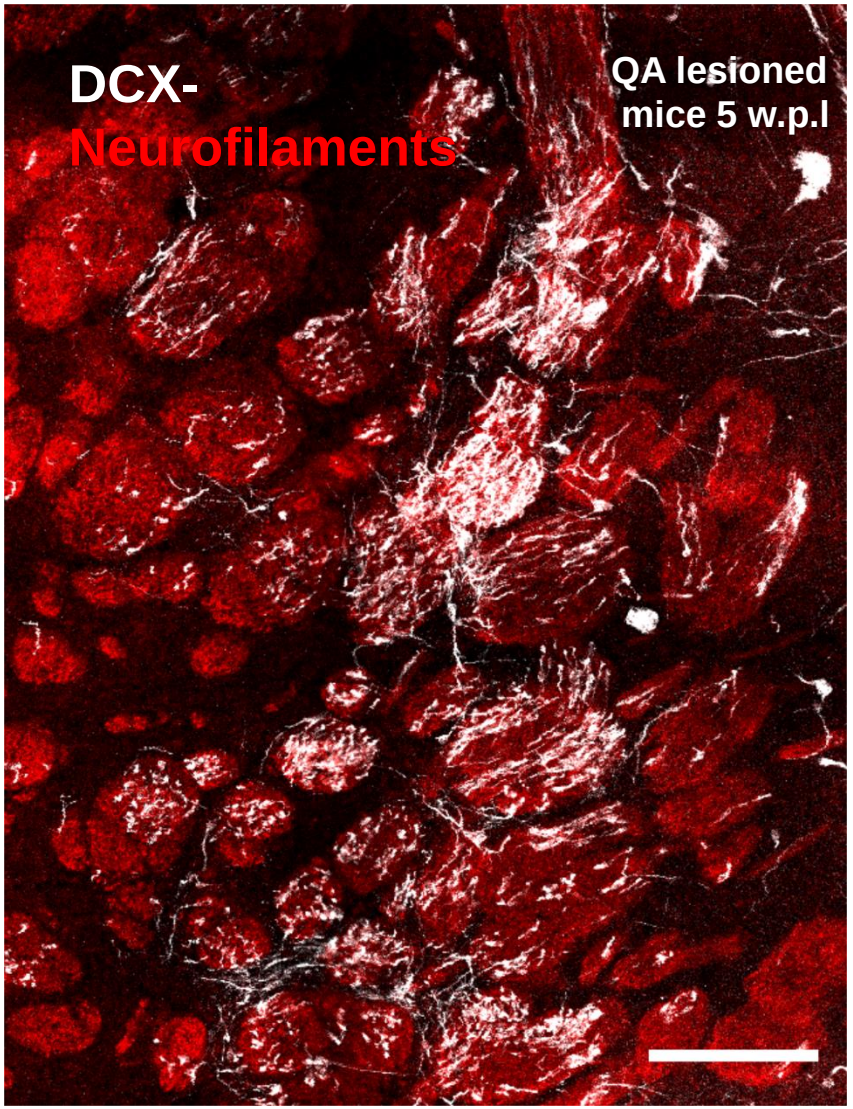
- **TRANSIENT EXISTENCE**

and retain the expression of DCX during their entire life

IDENTITY OF NEWBORN STRIATAL NEUROBLASTS



Neuroblasts show complex morphologies



DCX+ NEUROBLASTS POSSESS A STRONG TROPISM FOR INTERNAL CAPSULE BUNDLES

ARE NEW BORN NEURONS SINAPTICALLY CONNECTED?

MONOSINAPTING TRACING TECHNIQUE

1. Retrovirus encoding:

- RFP

- the EnvA receptor (TVA) for restricting primary RABV infection to newborn neurons as “starter” population

- the RABV glycoprotein, which is necessary for subsequent monosynaptic transfer (via transcomplementation) to their first-order presynaptic partners.

2. Rabies virus carrying the following modifications:

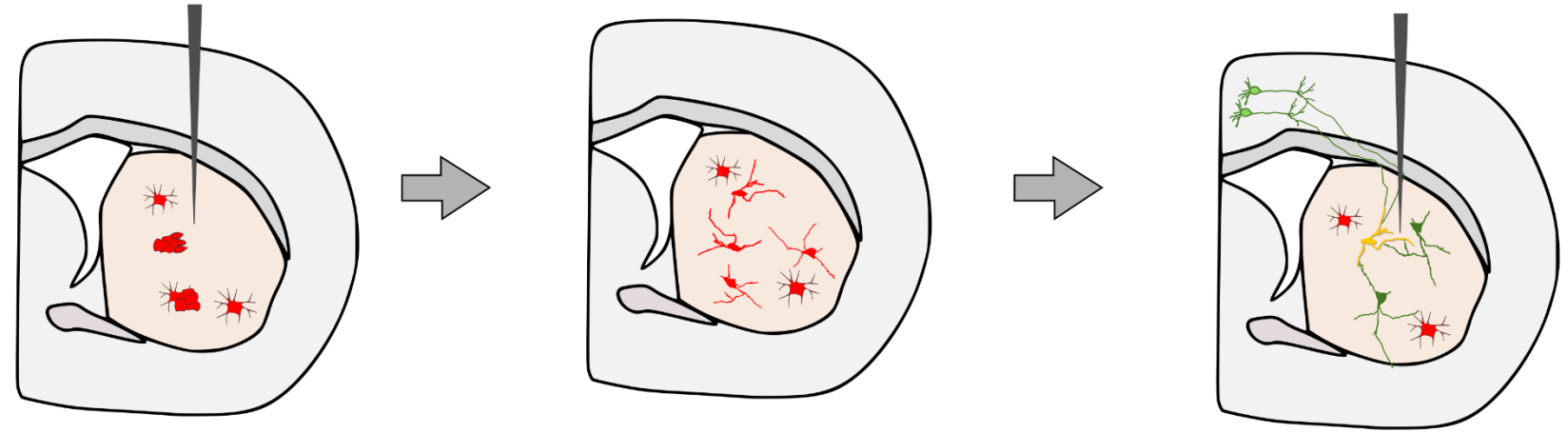
- Gene encoding for the GFP

- ASLV-A-pseudotyped in order to specifically infecting cells that express TVA receptor, a protein which is found in birds but not mammals;

-deletion of the rabies virus glycoprotein gene, required for transsynaptic spread

MONOSINAPTING TRACING TECHNIQUE

G-TVA Retro injection (RFP)
At 3 wpl, beginning of the neurogenic phase



RABV injection (GFP)

Target: dividing cells (RFP+)
- proliferating astrocytes,
- intermediate progenitors
- proliferating neuroblasts

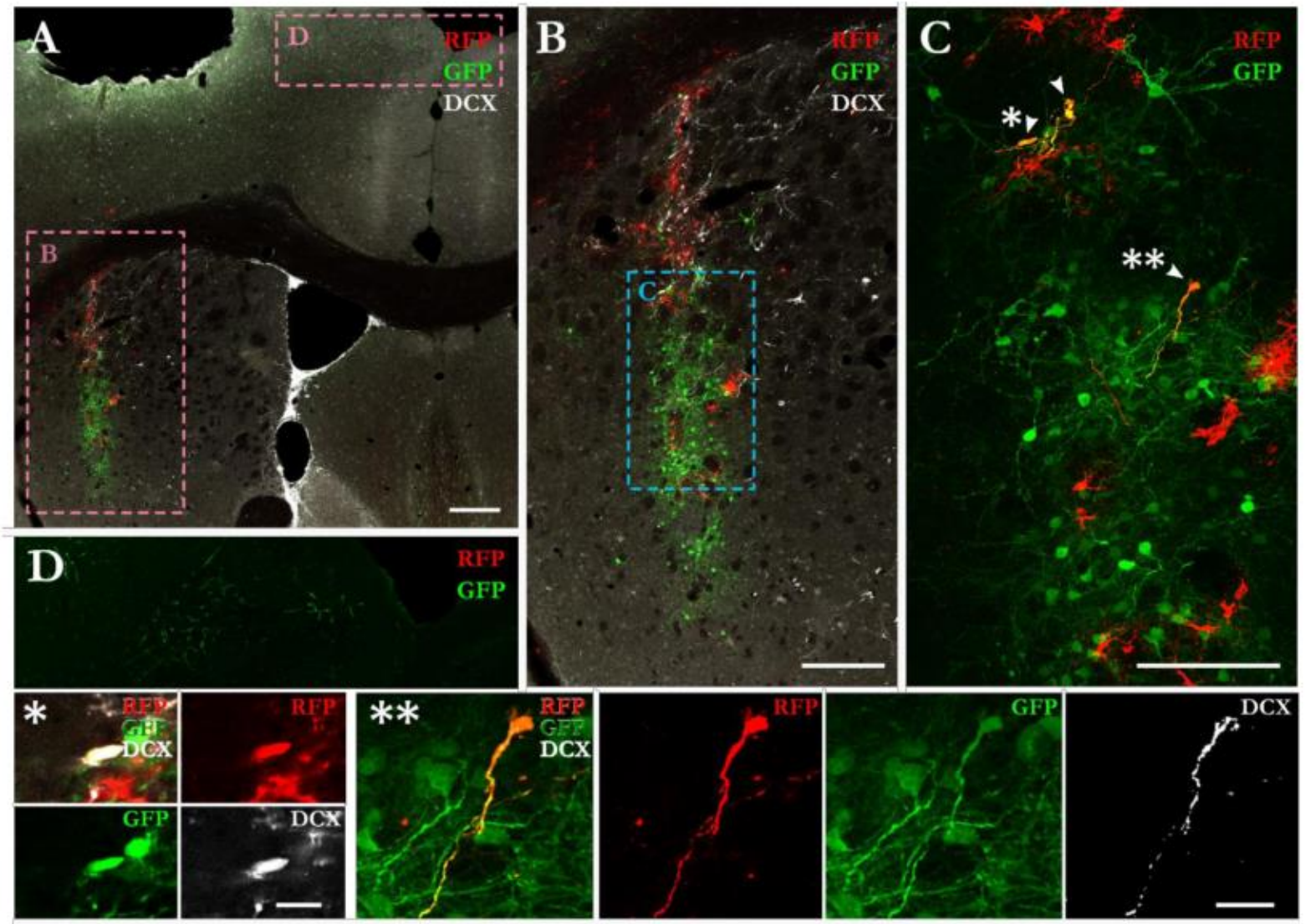
Target: cells pre-infected with the retrovirus (starter cell RFP+/GFP+)
- Striatal newborn neurons

From the starter cells the RABV spreads transynaptically to the first afferent, (Presynaptic GFP+ neuron

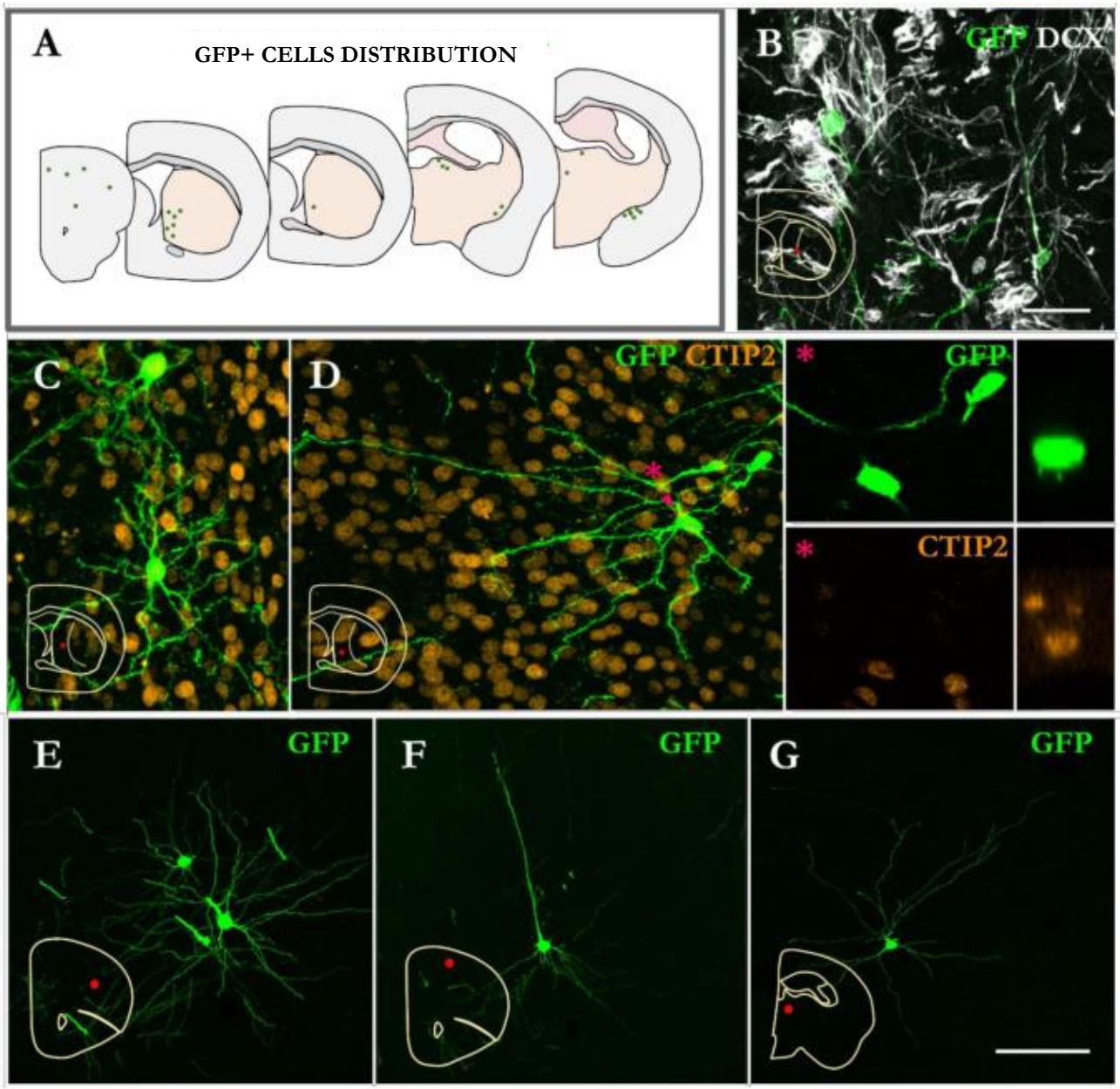
Red cells: cells infected only by the retrovirus

Yellow cells: starter cells
Green cells: neurons connected with the yellow cells.

NEWBORN NEURONS ARE CONNECTED TO BOTH LOCAL…



NEWBORN NEURONS ARE CONNECTED TO BOTH LOCAL AND LONG RANGE AFFERENTS



HYPOTHESIS

Striatal neuroblasts are:

- **A specific population of TRANSIENT NEURONS**
- **Involved in some forms of STRUCTURAL PLASTICITY, possibly including the remodeling of long-range connections.**

CONCLUSIONS

In adult mammals glial cells, both SVZ progenitors and parenchymal astrocytes, react after injuries by increasing their proliferation and producing new neurons.

Further studies, will be required to better understand the the fate and the role of the newly generated neurons

Three main hypothesis (not mutually exclusive):

1) **ineffective attempt to replace the degenerate neurons**
2) **misdirected OB neurons**
3) **specific population of neurons characterized by transient life and involved in some form of plasticity or neuroprotection**