A Widespread Neurogenic Potential of Neocortical Astrocytes Is Induced by Injury

--- Journal Club

Xiangjie Zhao

Sep 3, 2020

FRISÉN LAB

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RESEARCH FOCUS

We are interested in the role of stem cells in cell turnover in healthy and pathological situations. We study the identity of stem cells and the organization of cellular lineages, the molecular regulation of cell production and the role of cell renewal in homeostasis and pathological situations. Many of our projects focus on stem cells in the brain and spinal cord and adult neurogenesis.

We are also interested in cell renewal in the heart and use the intestine as a stem cell and cancer model system. We have developed a method to study cell turnover by analyzing the integration of 14C derived from nuclear bomb tests in DNA, and use this to assess cell renewal in humans.

Background

1. Rbpj-κ Deletion and Stab Wound Injury Trigger Neurogenesis in the Somatosensory Cortex
2. Notch Signaling Represses a Neurogenic Transcriptional Program in Cortical Astrocytes
3. Reconstruction of Transcriptional Dynamics during Astrocyte Neurogenesis
4. Reconstruction of Gene-Regulatory Network Activity Identifies Transcriptional Programs Driving Cortical Neurogenesis
5. Cortical Neurogenesis Recapitulates Canonical Subventricular Zone Neurogenesis

Summary and discussion

The adult mammalian brain has limited regenerative capacity after neuronal loss

Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke

Maryam Faiz [1], Nadia Sachewsky [2, 5], Sergio Gascón [3, 4], K.W. Annie Bang [1], Cindi M. Morshead [2, 5] ✉, Andras Nagy [1, 6] ✉

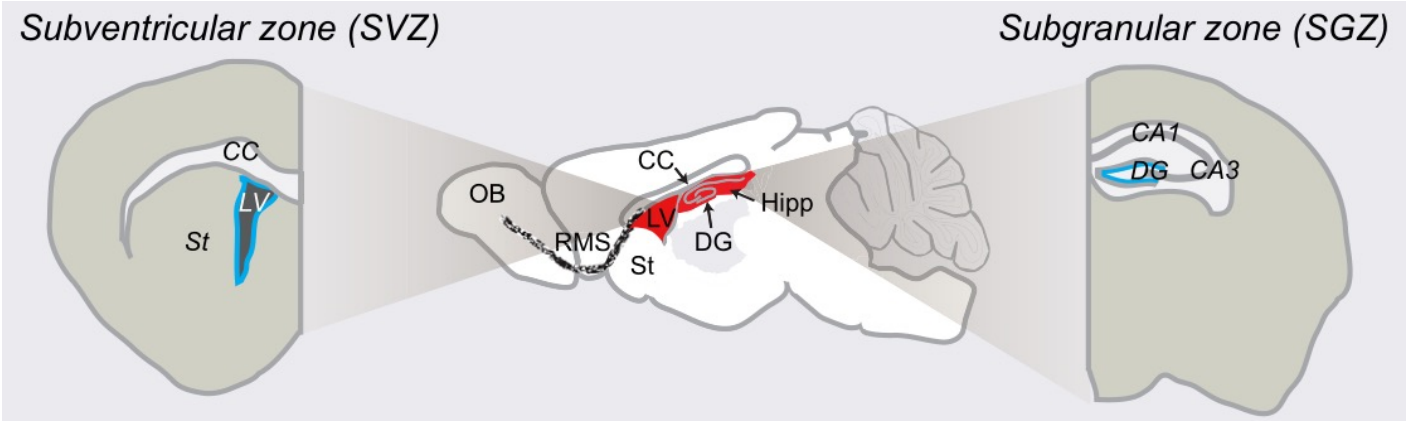
generate glia

Neuronal replacement from endogenous precursors in the adult brain after stroke

Andreas Arvidsson ✉, Tove Collin, Deniz Kirik, Zaal Kokaia & Olle Lindvall

Nature Medicine **8**, 963–970(2002) | Cite this article

contribute to replacement of a minor portion of neurons lost to injury



Allison M. Bond et al., Cell Stem Cell (2015)

Parenchymal astrocytes represent a potential novel source of latent neurogenic cells

Article

Reactive Glia in the Injured Brain Acquire Stem Cell Properties in Response to Sonic Hedgehog

Swetlana Sirko [1, 4, 14], Gwendolyn Behrendt [1, 14], Pia Annette Johansson [4], Pratibha Tripathi [4, 15], Marcos Romualdo Costa [4, 16], Sarah Bek [1, 4], Christophe Heinrich [1], Steffen Tiedt [1], Dilek Colak [4, 5], Martin Dichgans [6, 7], Isabel Rebekka Fischer [6], Nikolaus Plesnila [6, 7], Matthias Staufenbiel [8], Christian Haass [2, 3, 7], Marina Snapyan [9], Armen Saghatelyan [9], Li-Huei Tsai [10, 11], André Fischer [12] ... Magdalena Götz [1, 4, 7]

Astrocytes isolated from the injured cortex display neural stem cell properties in vitro (Sirko et al., 2013).

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,†]

[1]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden.

[2]Lund Stem Cell Center, University Hospital, SE-221 84 Lund, Sweden.

[3]Division of Translational Cancer Research, Lund University, SE-223 63 Lund, Sweden.

[†]Corresponding author. E-mail jonas.frisen@ki.se

[*] These authors contributed equally to this work.

– Hide authors and affiliations

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

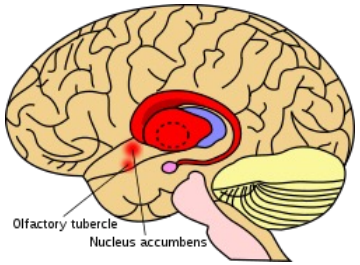
Giulia Nato, Alessia Caramello, Sara Trova, Valeria Avataneo, Chiara Rolando, Verdon Taylor, Annalisa Buffo, Paolo Peretto, Federico Luzzati

Development 2015 142: 840-845; doi: 10.1242/dev.116657

In mice subjected to middle cerebral artery occlusion (Magnusson et al., 2014) or excitotoxic damage (Nato et al., 2015), astrocytes give rise to new neurons in vivo

Questions to answer

Striatum (red)



1. **Previous study show that, even when Rbpj-κ is depleted from astrocytes throughout the brain, neurogenesis occurs almost exclusively in the striatum, raising questions about how widespread the neurogenic potential of astrocytes is.**

2. **The molecular cascade that drives the lineage fate transition is, however, unclear.**

3. **It remains to be determined whether the transcriptional programs required for parenchymal astrocytes to generate adult-born neurons share similarities with canonical neurogenic processes.**

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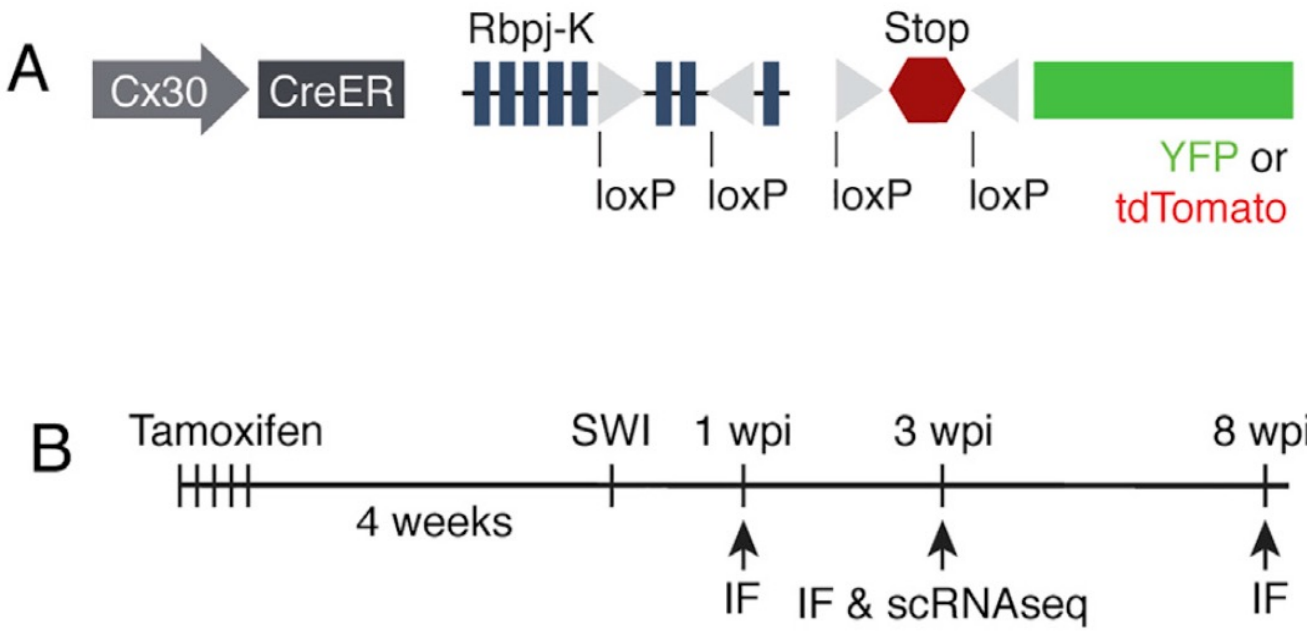
Summary and discussion

Transgenic strategy and experimental timeline



Cx30: astrocyte marker

Rbpj-K: transcriptional regulator that plays a central role in Notch signaling

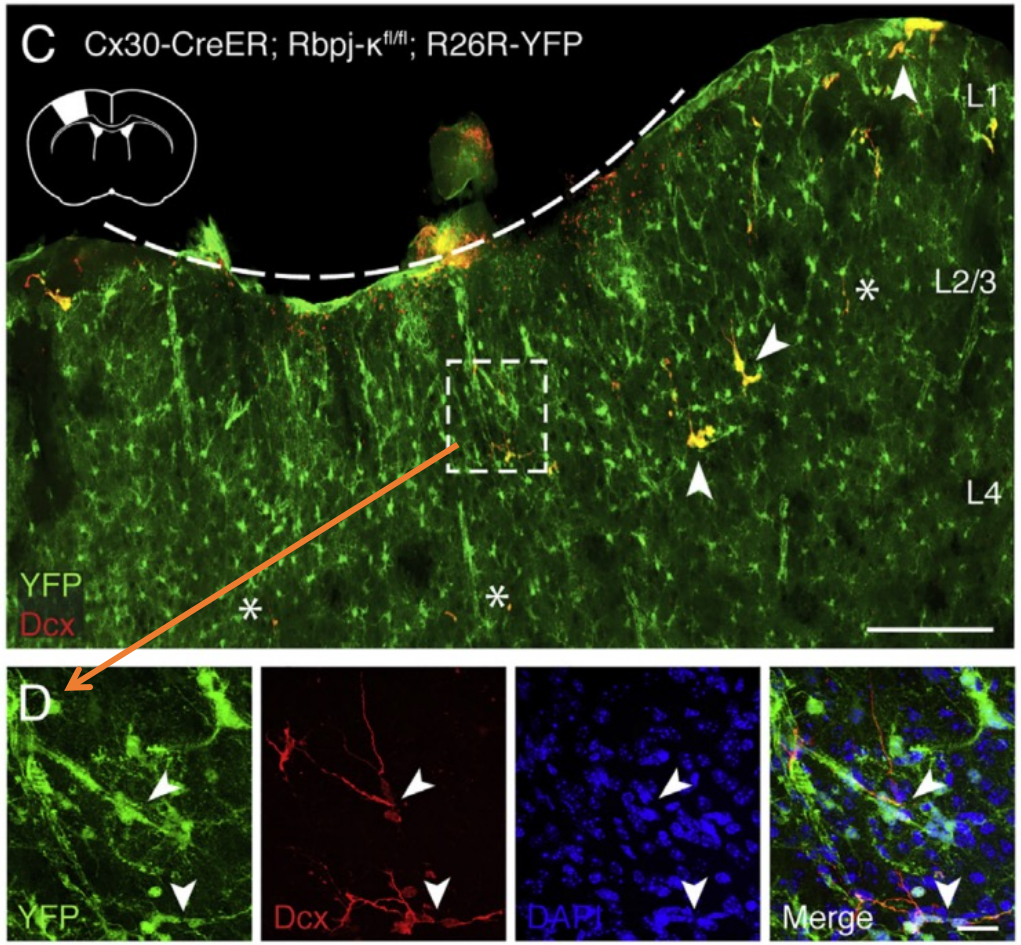
stab wound injury (SWI)

week post-injury (wpi)

selective ablation of Rbpj-κ and reporter expression in astrocytes and their progeny

Rbpj-κ KO and stab wound injury result in astrocytes initiating a neurogenic program

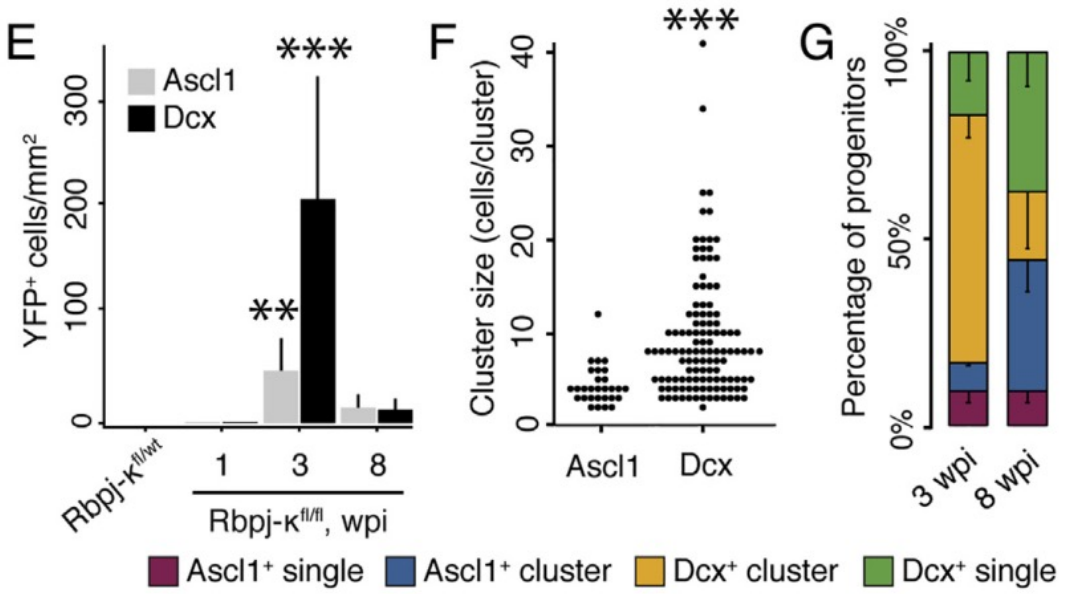
Damaged region (dashed line)
Dcx-expressing neural progenitors
proliferative clusters (star) or single cells (arrow)



25 μm

an example of branched neuroblasts

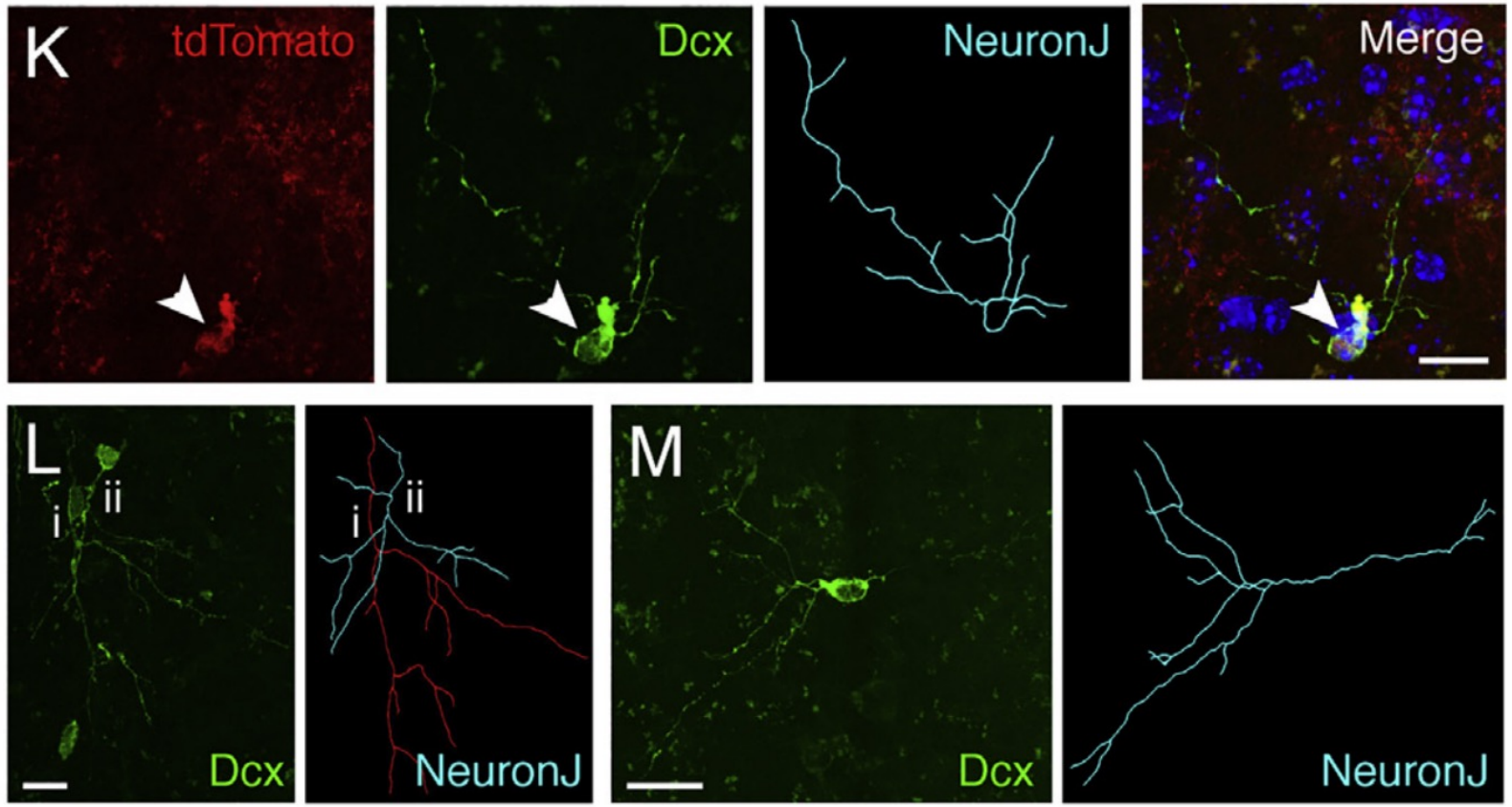
Ascl1: transit-amplifying cell marker
Dcx: neuroblast marker



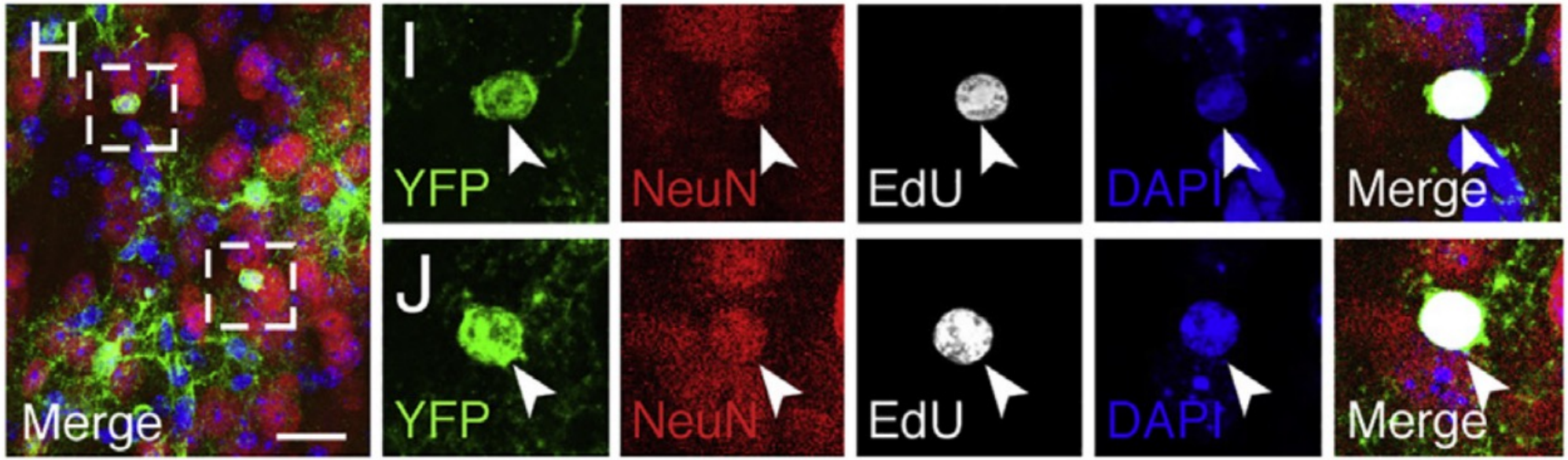
first appeared 1 wpi,
peaked at 3 wpi

Expansion of Ascl-expressing transit-amplifying cell

Examples of Dcx-expressing cells with a complex morphology indicative of neuronal maturation



EdU in YFP/NeuN double positive cells indicated that the mature neurons were generated by astrocytes that had divided after injury



NeuN (Neuronal Nuclei): mature neuron marker

Mice subjected to stab wound injury were given EdU starting after the surgical procedure

Are transit-amplifying cells and neuroblasts produced locally?

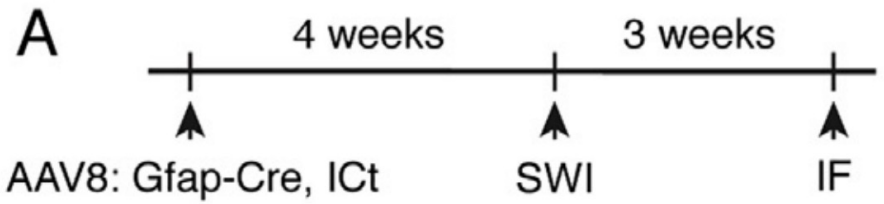
Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke

Maryam Faiz [1], Nadia Sachewsky [2, 5], Sergio Gascón [3, 4], K.W. Annie Bang [1], Cindi M. Morshead [2, 5] ✉, Andras Nagy [1, 6] ✉

NSCs from SVZ can migrate to injured region
and differentiate into astrocytes.

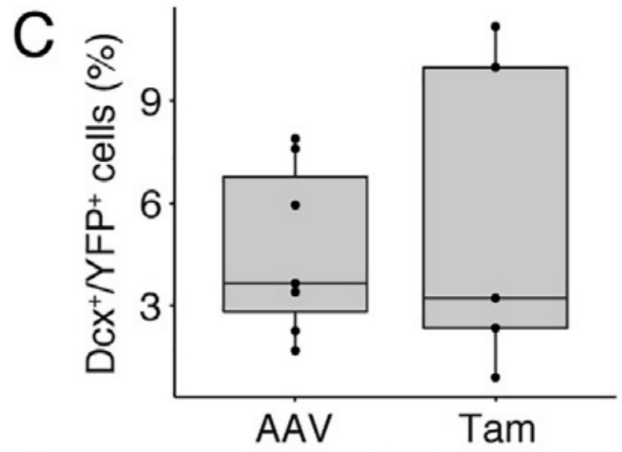
NSCs also express Cx30. Rbpj-κ KO astrocytes in injured cortex may be from SVZ.
Therefore, they could not exclude that the neuroblasts observed in the cortex had migrated from the SVZ.

Local cortical origin of the neural progenitors



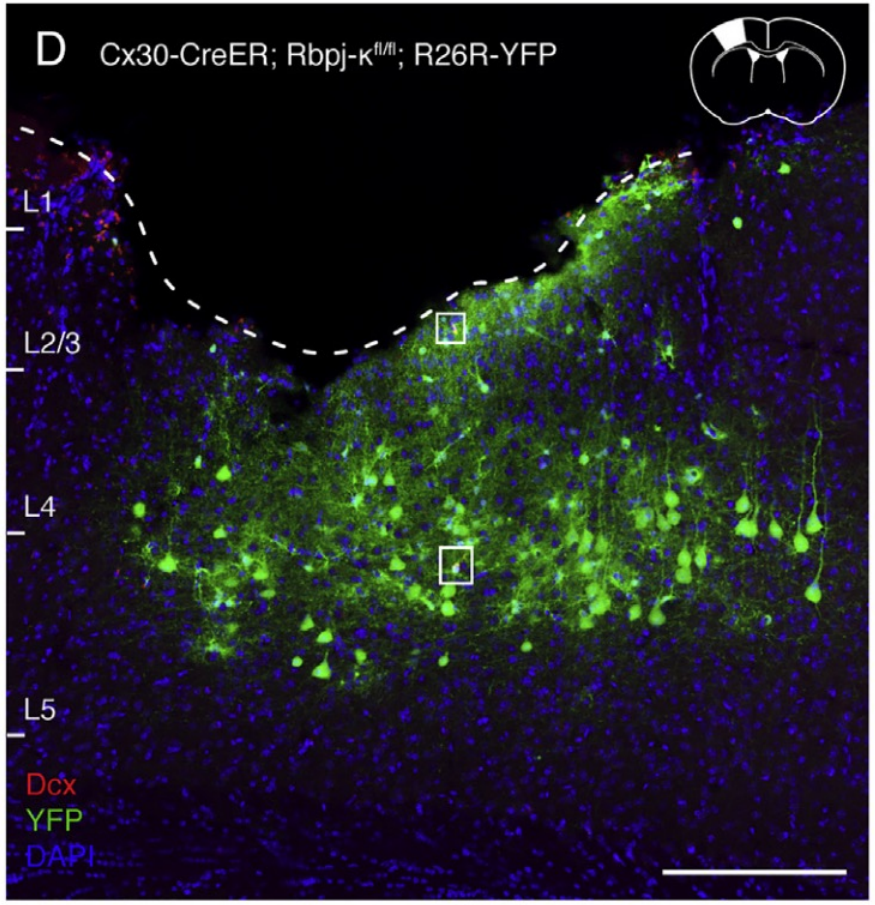
Gfap: astrocyte marker

Focal injection of anadeno-associated virus carrying Cre under the Gfap promoter in Rbpj−κ$^{fl/fl}$ mice

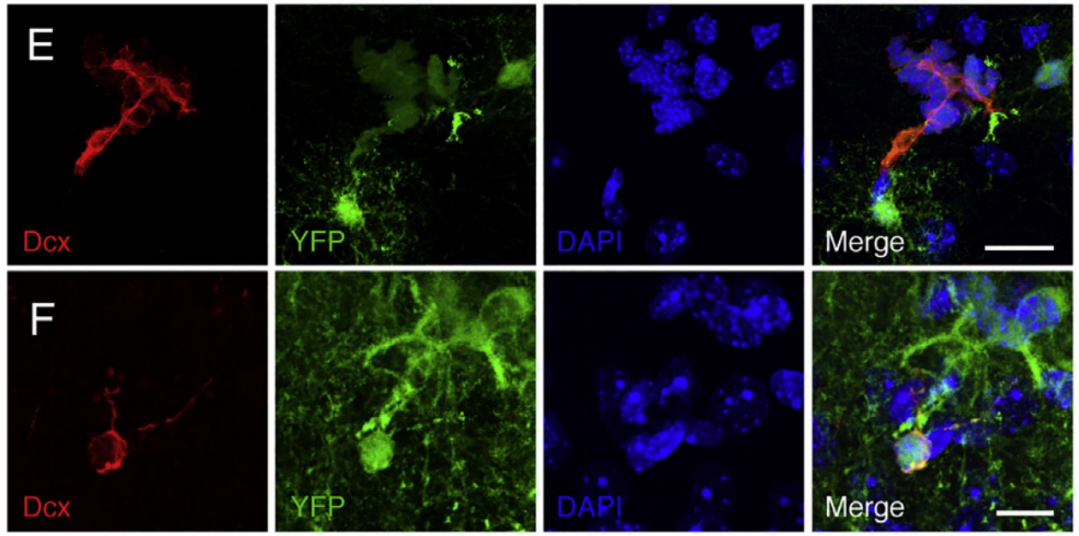


The percentage of YFP + cells expressing the neurogenic marker were comparable between local and systematic recombination

Local cortical origin of the neural progenitors



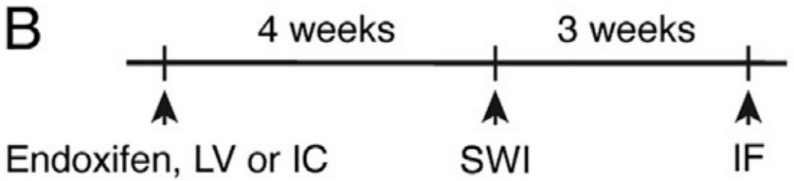
magnified



10 μm

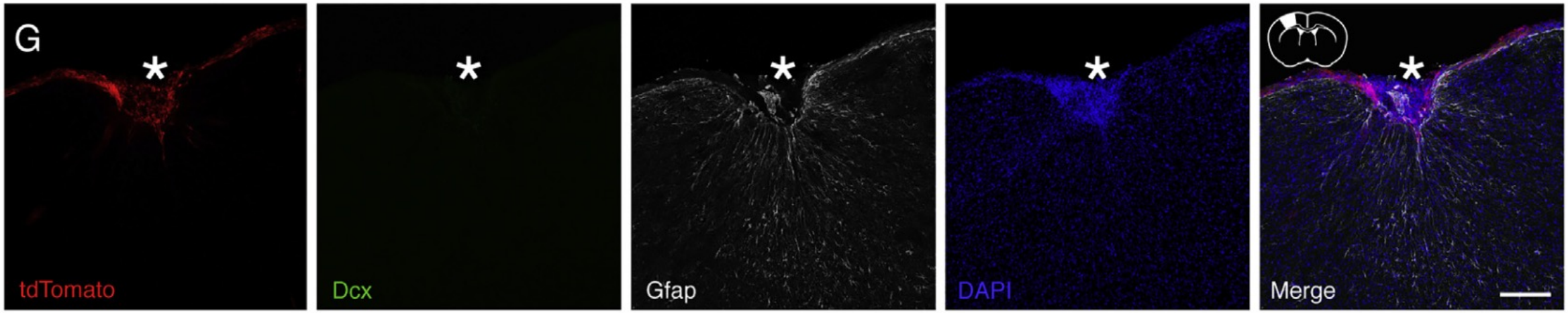
SVZ are not a source of neurogenic cells

Like astrocytes, NSCs and VLMCs express Cx30
(De Bock et al., 2014)



Delivery of endoxifen to the lateral ventricle (LV) or the cisterna magna (IC) was used to demonstrate the lack of contribution of SVZ and vascular leptomeningeal cells (VLMCs), respectively

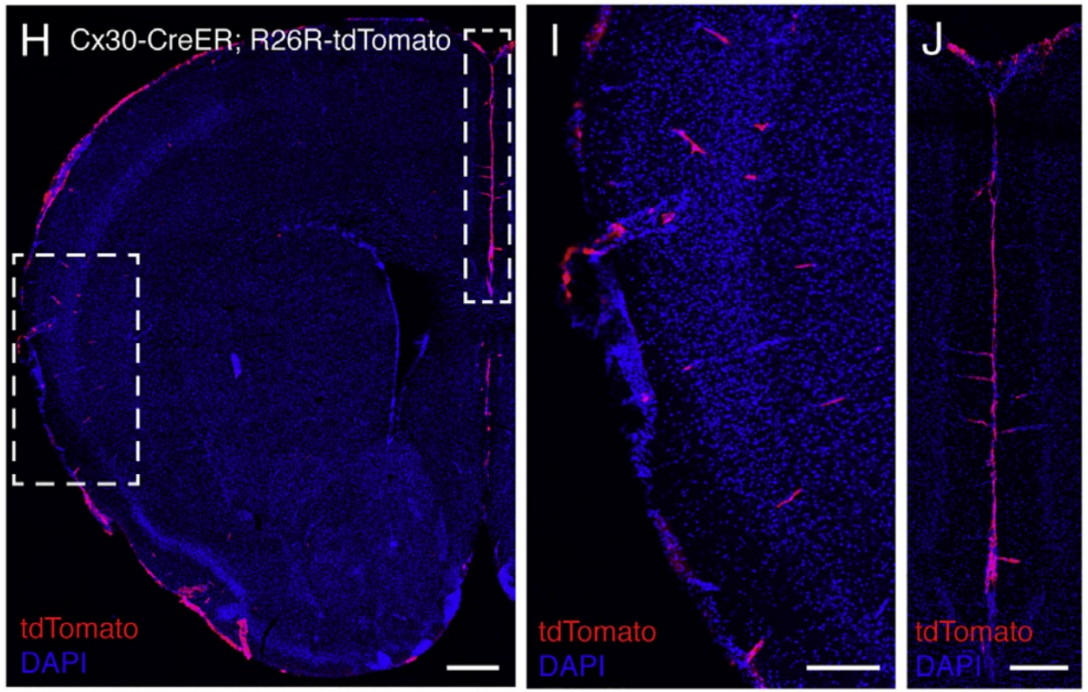
endoxifen, an active metabolite of tamoxifen



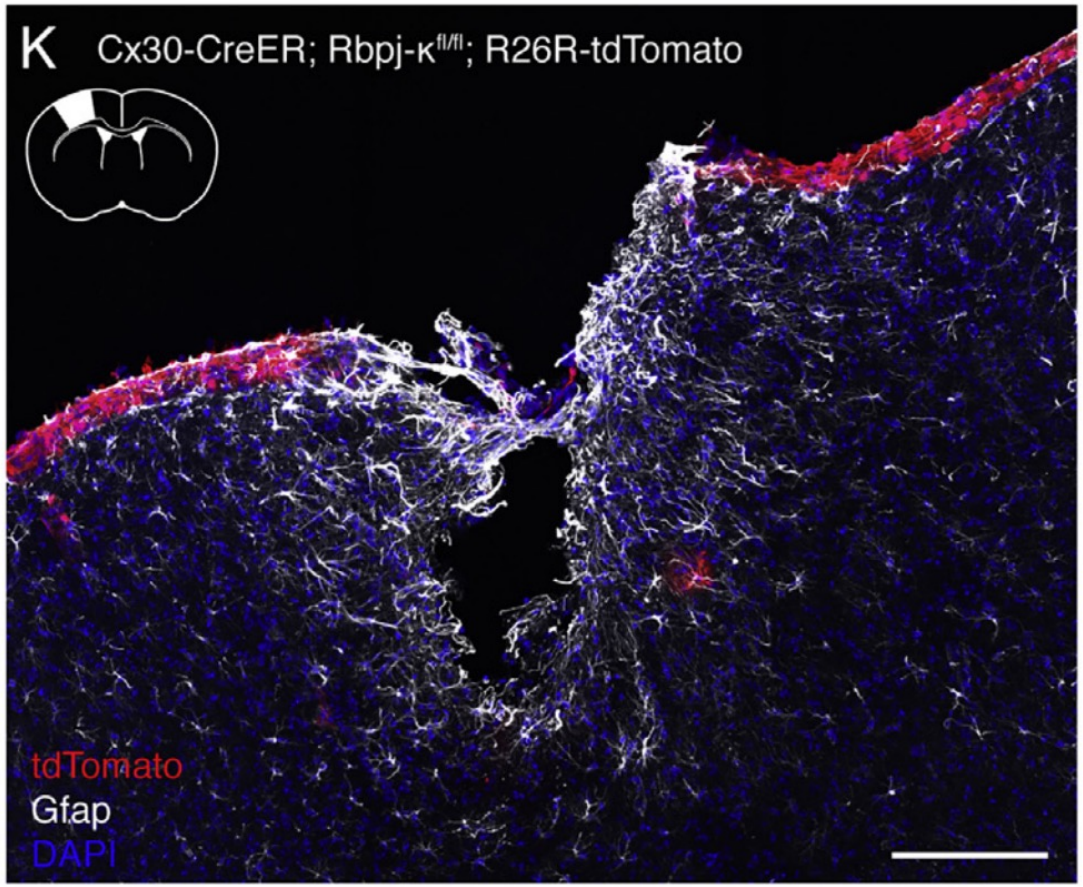
cortical lesion site (*)

KO Rbpj in SVZ

VLMCs are not a source of neurogenic cells



VLMCs surrounding the cortex and penetrating the parenchyma alongside blood vessels become recombined with this injection



Absence of Ascl1- or Dcx-expressing cells in injured cortical regions

KO Rbpj in VLMC

To summarize, transit-amplifying cells and neuroblasts identified before are produced locally in somatosensory cortex.

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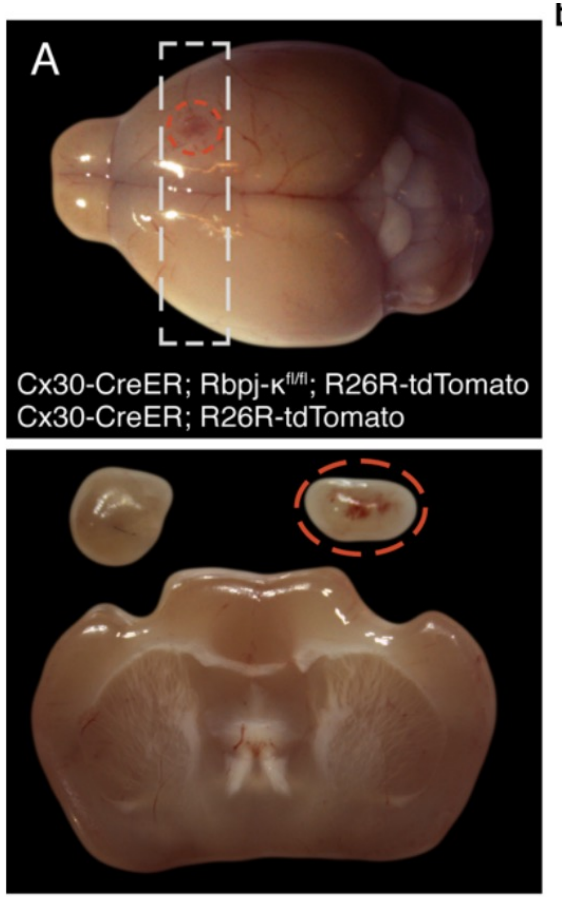
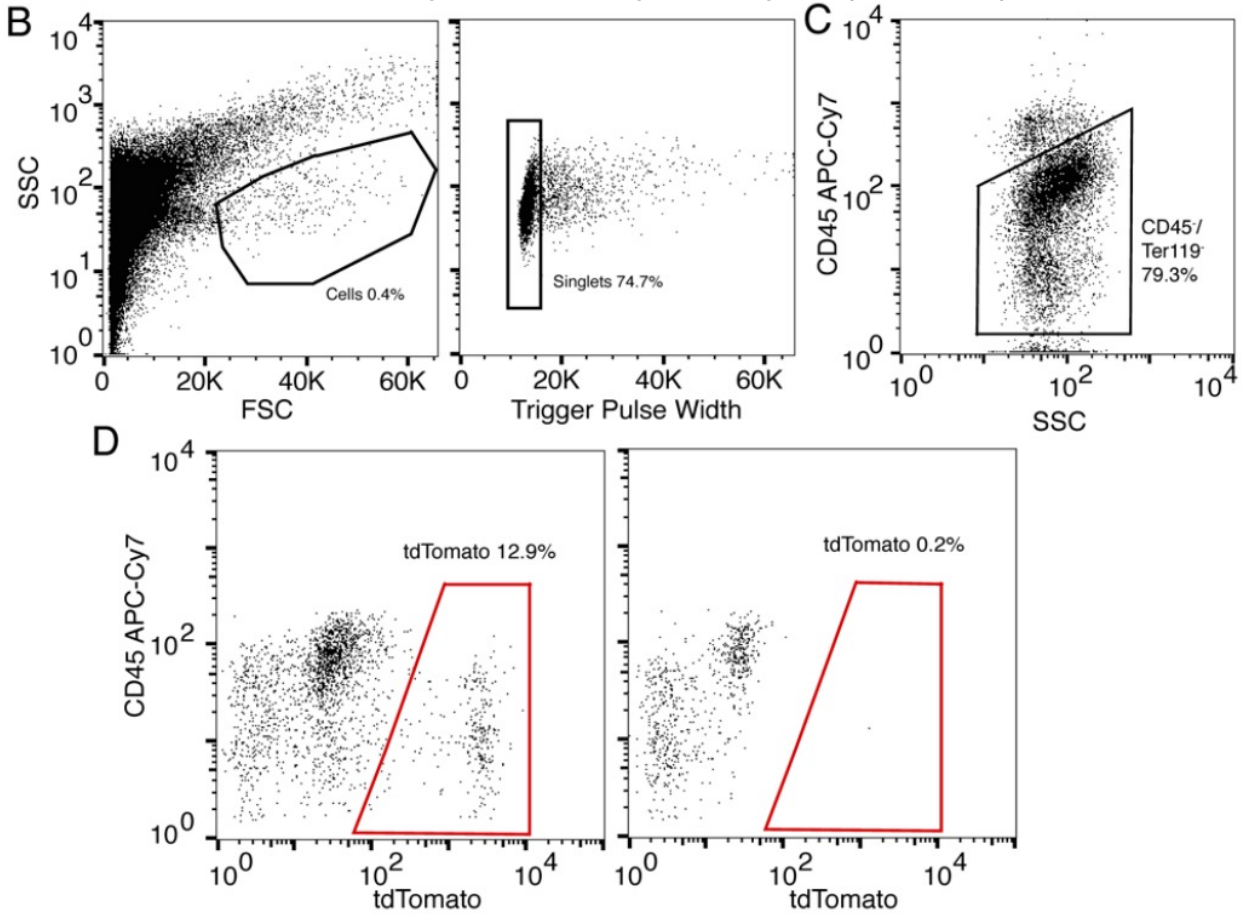
Summary and discussion

Sample preparation for scRNA-Seq



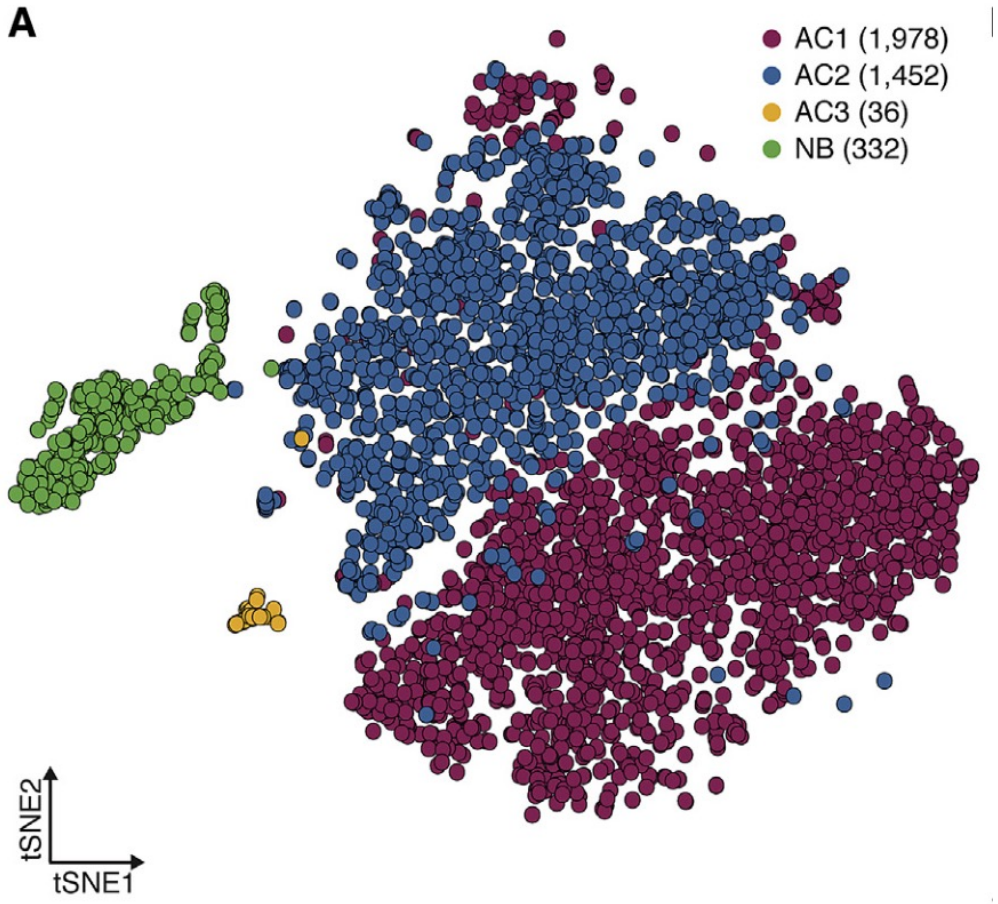
They dissected portions of the somatosensory cortex subjected to stab wound injury and healthy contralateral cortices of wild-type and Rbpj-κ KO mice

Remove leukocyte and erythrocyte (红细胞)

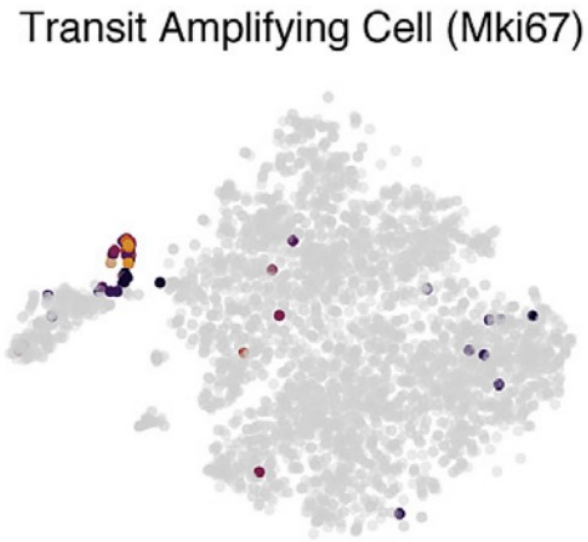


Using flow cytometry, they then sorted tdTomato positive cells (i.e., Rbpj-κ KO astrocytes and their progeny) and analyzed their transcriptome

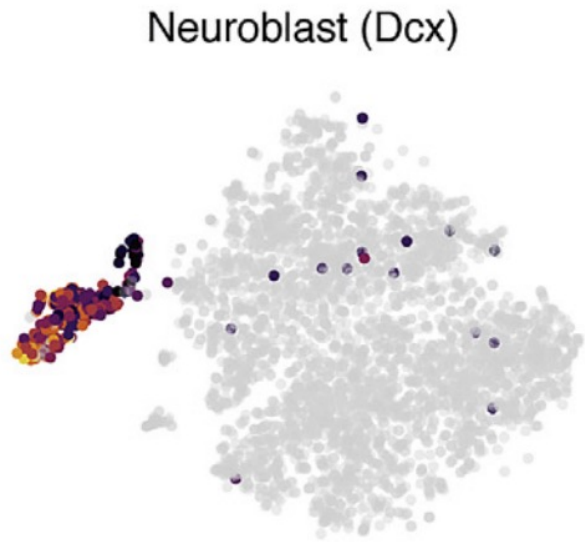
Clustering identifies three astrocyte clusters (AC1–AC3) that segregate from the neurogenic progeny



AC: astrocyte
NB: neuroblast

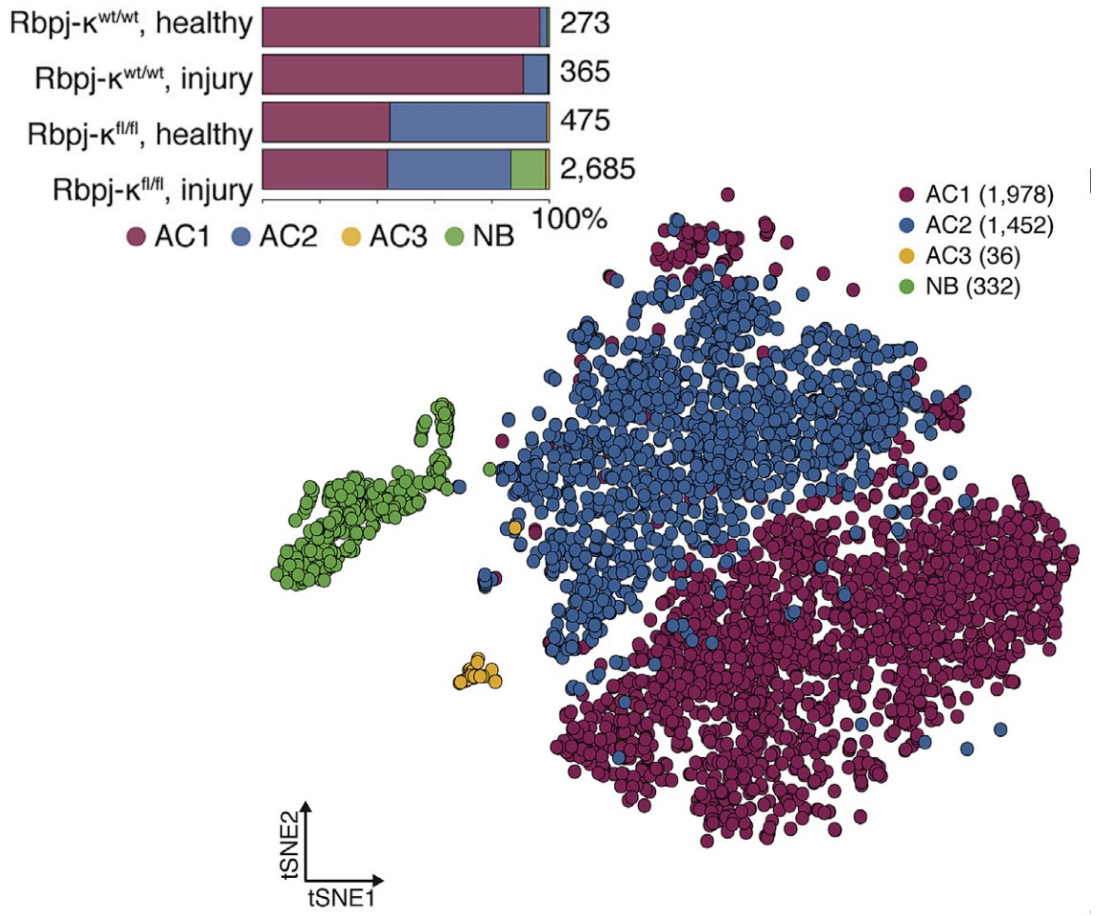


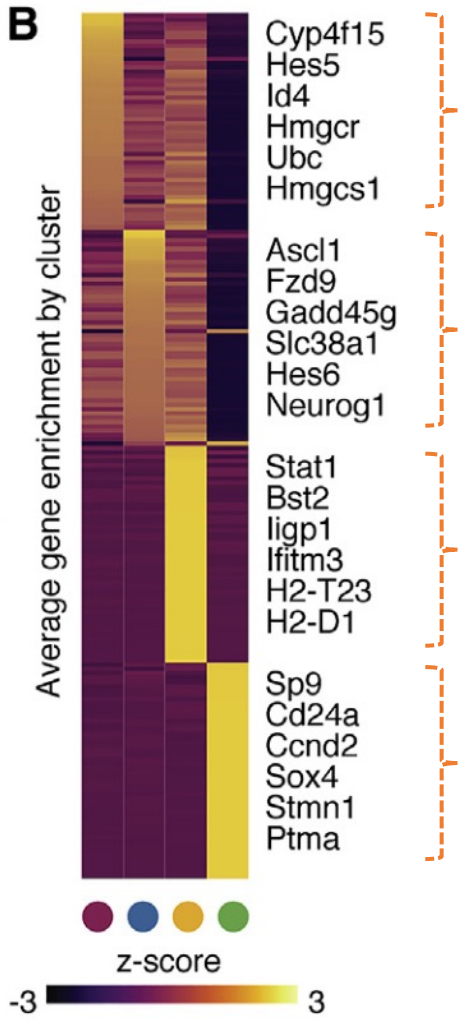
cell cycle gene



neuronal specification gene

Notch represses a neurogenic transcriptional program in cortical astrocytes





Notch signaling genes;
inhibitors of neurogenesis;
cholesterol biosynthesis

genes typically inhibited by notch pathway;
neurogenic genes

reactive and immune response-related genes

cell cycle and neuronal specification-related genes

Initiation of the neurogenic program is widespread throughout the cortical layers

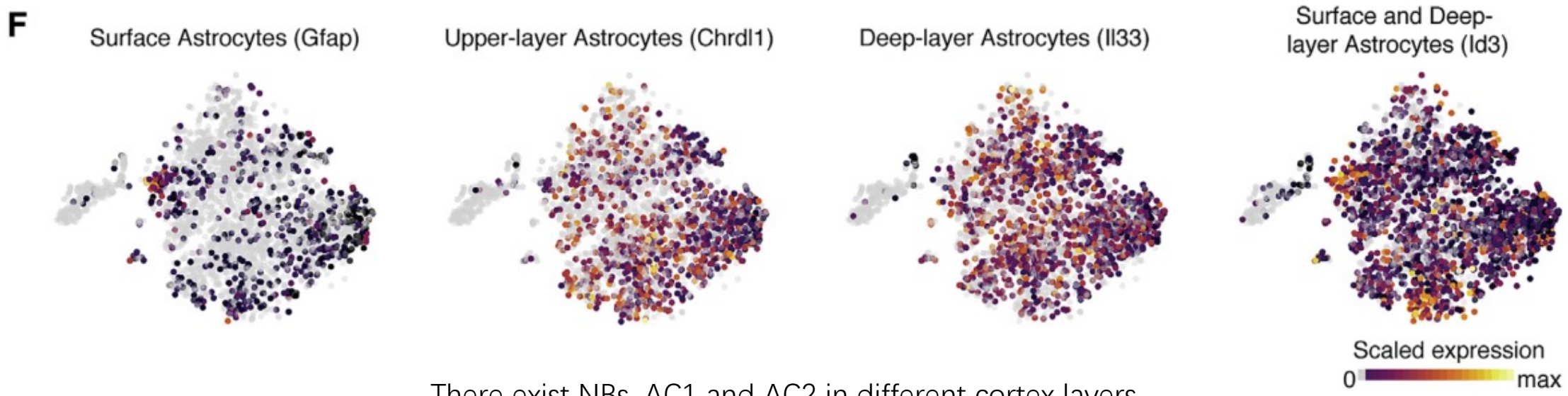
Article | Published: 16 March 2020

Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map

Omer Ali Bayraktar, Theresa Bartels, Staffan Holmqvist, Vitalii Kleshchevnikov, Araks Martirosyan, Damon Polioudakis, Lucile Ben Haim, Adam M. H. Young, Mykhailo Y. Batiuk, Kirti Prakash, Alexander Brown, Kenny Roberts, Mercedes F. Paredes, Riki Kawaguchi, John H. Stockley, Khalida Sabeur, Sandra M. Chang, Eric Huang, Peter Hutchinson, Erik M. Ullian, Martin Hemberg, Giovanni Coppola, Matthew G. Holt, Daniel H. Geschwind & David H. Rowitch

Nature Neuroscience **23**, 500–509(2020) | Cite this article

Recent studies have identified markers of astrocytes in different cortical layers



There exist NBs, AC1 and AC2 in different cortex layers

Questions to answer

So can astrocytes across different layers of cortex

1. **Previous study show that, even when Rbpj-κ is depleted from astrocytes throughout the brain, neurogenesis occurs almost exclusively in the striatum, raising questions about how widespread the neurogenic potential of astrocytes is.**

2. The molecular cascade that drives the lineage fate transition is, however, unclear.

3. It remains to be determined whether the transcriptional programs required for parenchymal astrocytes to generate adult-born neurons share similarities with canonical neurogenic processes.

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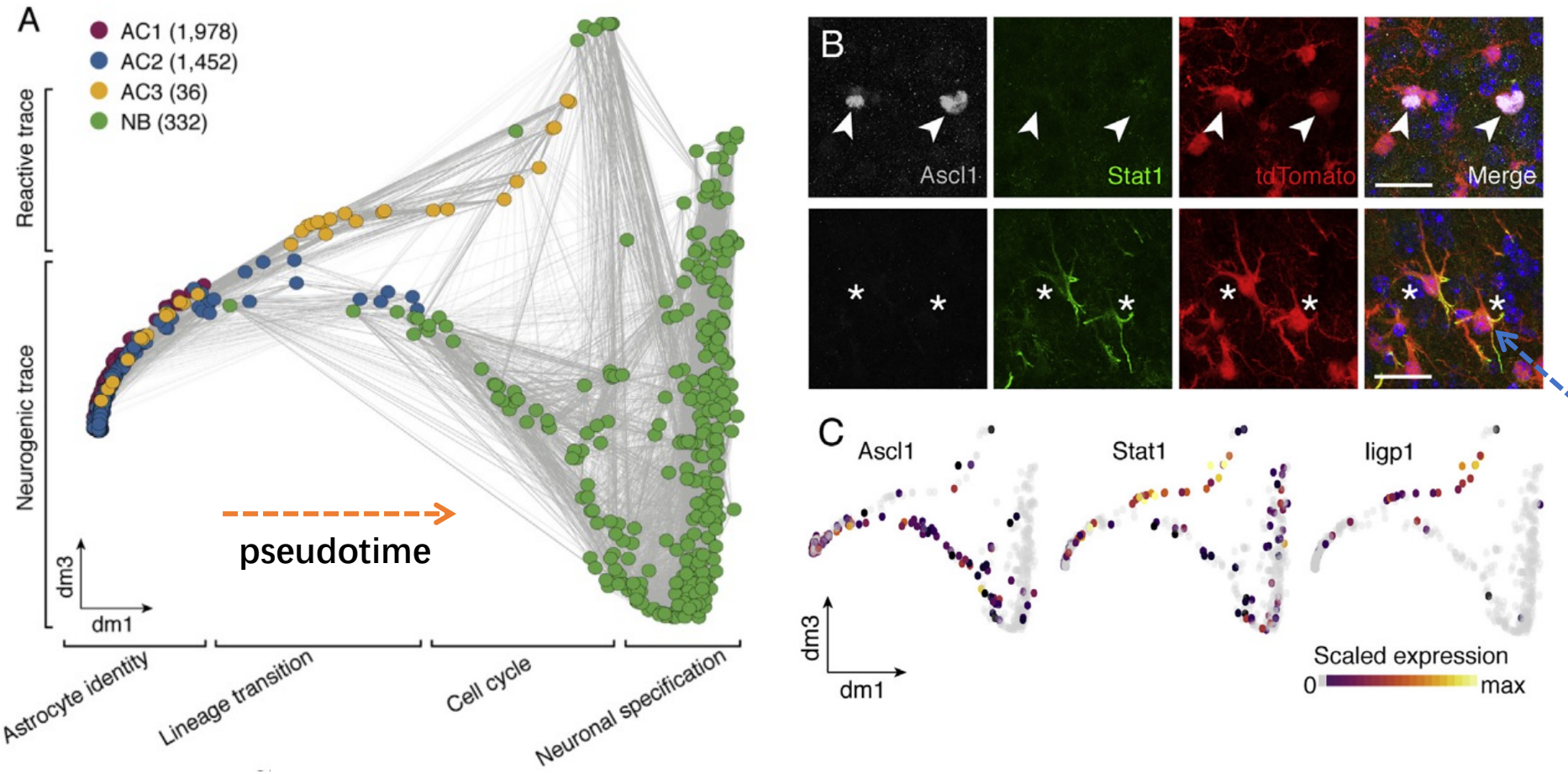
Summary and discussion

Reconstruction of an astrocyte neurogenic trajectory

Diffusion map

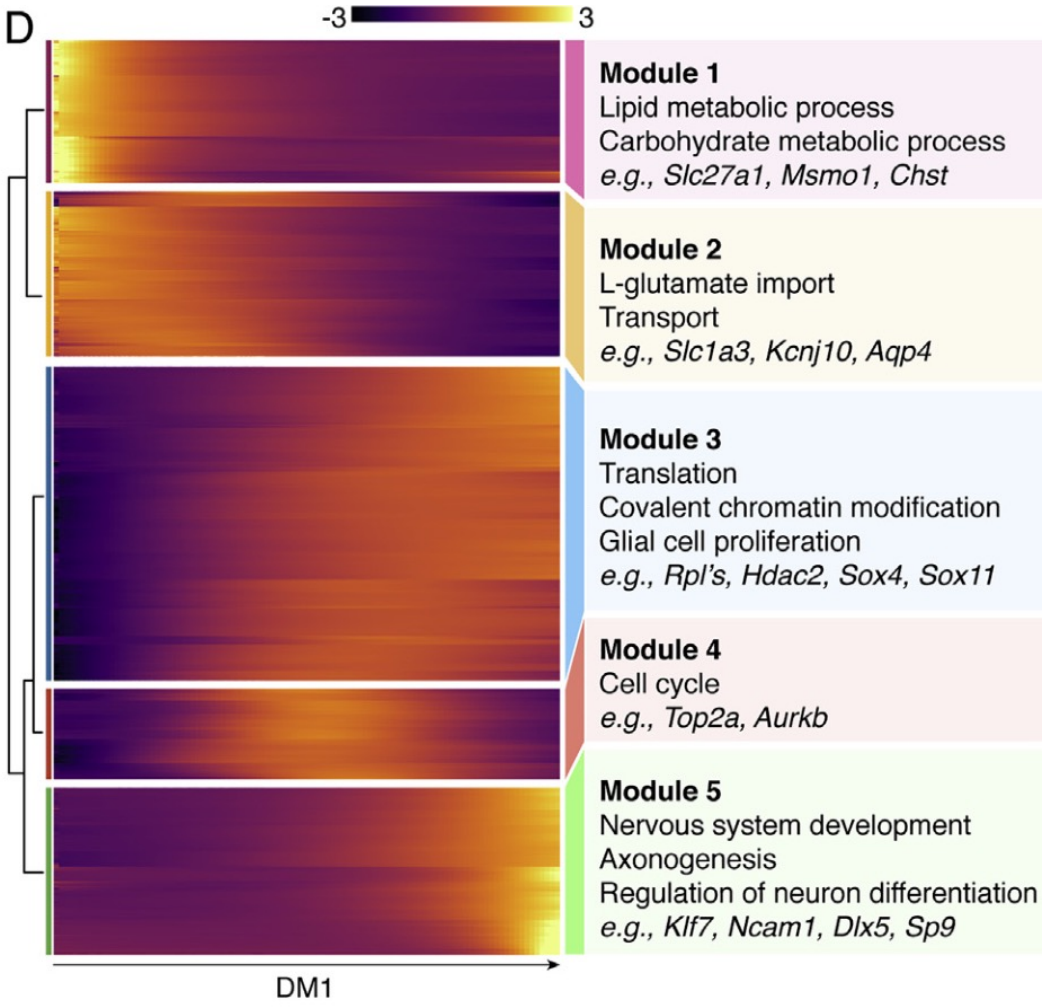


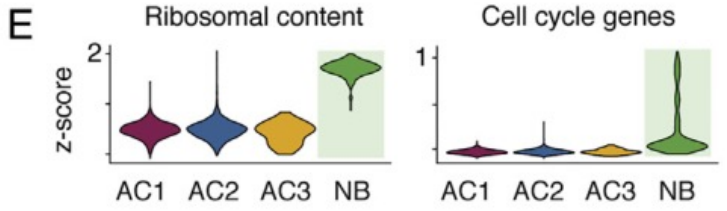
Ascl1 and Stat1 don't express together, confirming the 2 separated trajectories

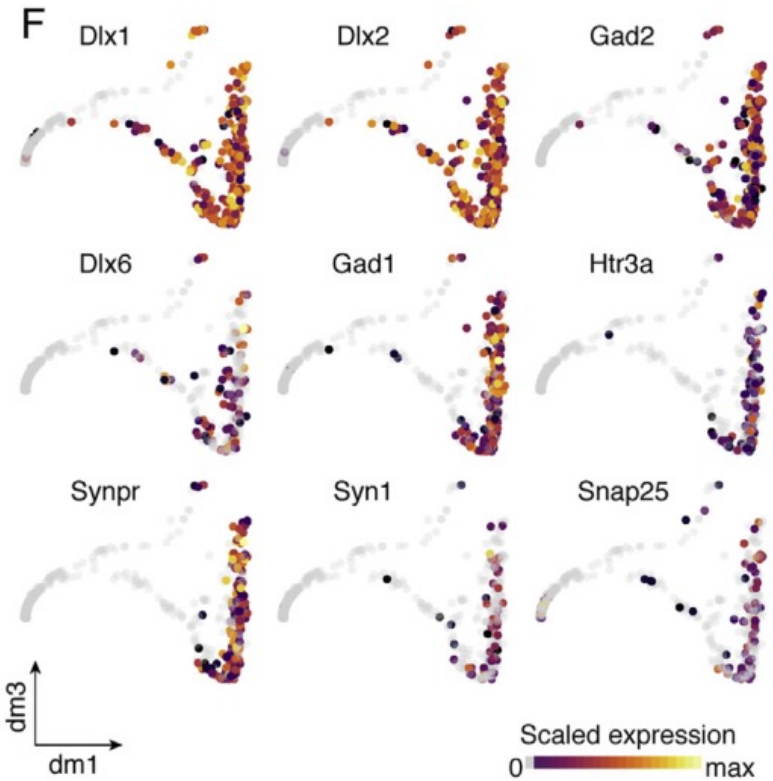
Stat1 positive astrocytes present hypertrophic morphologies typical of reactive glia

1. Wildtype asctrocytes (AC1) were polarized at one end of the pseudotemporal scale
2. Some of AC2 progressed toward TAP cells and NBs, spreading along the x axis
3. Some of AC3 formed a separate branch that did not progress toward neuroblasts

Differential expression analysis across pseudotime identified genes that dynamically expressed along the neurogenic path







indicative of a GABAergic interneuronal phenotype

key GABAergic signaling components

synapse formation and maintenance

maturing

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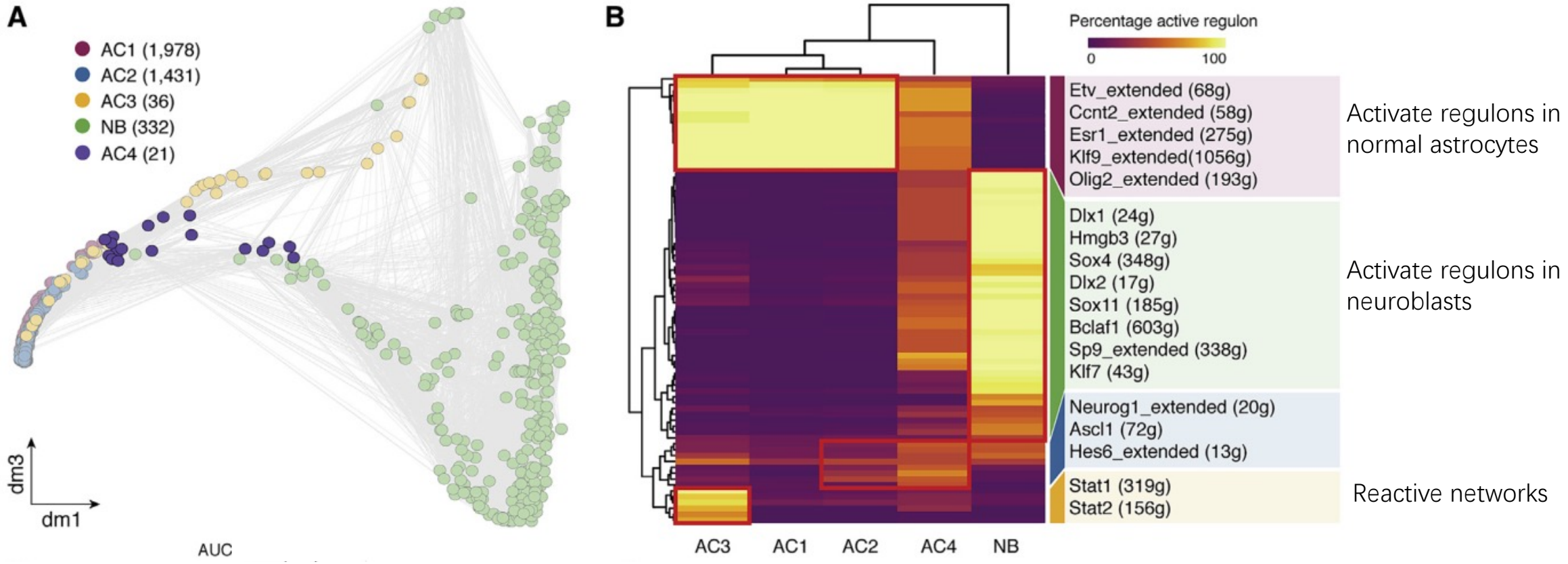
Summary and discussion

Reconstruction of gene-regulatory network activity identifies transcriptional programs driving cortical neurogenesis

Regulon format: TF (number of target genes)



Activate regulons in normal astrocytes

Activate regulons in neuroblasts

Reactive networks

Subsetting AC2 according to maximal pseudotime of AC1
This cluster is at the time of transition from astrocytes to the neuronal lineage

Identification of 206 regulons using SCENIC
Clustering regulons by their activate percentages in each cell type

Questions to answer

1. Previous study show that, even when Rbpj-κ is depleted from astrocytes throughout the brain, neurogenesis occurs almost exclusively in the striatum, raising questions about how widespread the neurogenic potential of astrocytes is.

They identified transcriptomic programs driving astrocytes transiting towards neuron (after Rbpk-κ KO and SWI)

2. **The molecular cascade that drives the lineage fate transition is, however, unclear.**

3. It remains to be determined whether the transcriptional programs required for parenchymal astrocytes to generate adult-born neurons share similarities with canonical neurogenic processes.

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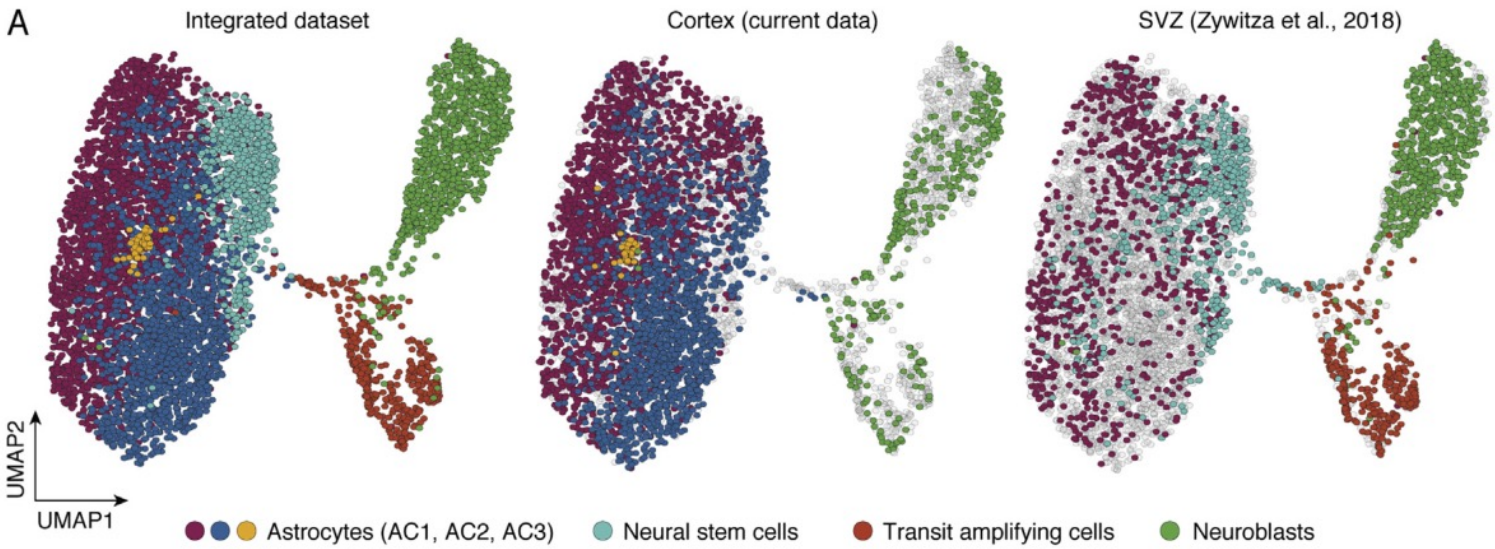
5. **Cortical Neurogenesis Recapitulates Canonical Subventricular Zone Neurogenesis**

Summary and discussion

Dataset alignment of cortex and SVZ reveals convergent neurogenic programs



They then clustered integrated data.

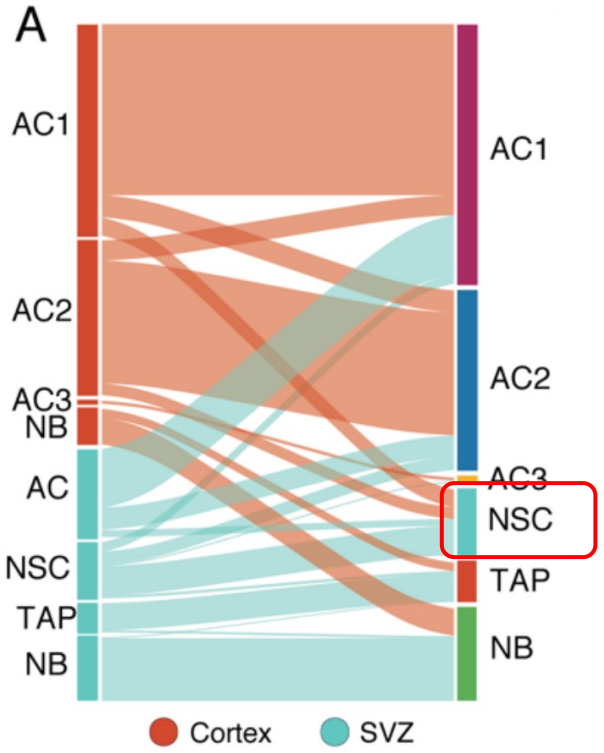
Integrated data maintained a similar partition among astrocyte subgroups and clusters of quiescent neural stem cells, transit amplifying cells, and neuroblasts

They aligned their dataset to a published scRNA-seq experiment encompassing SVZ cells using data integration algorithm

Data integration identified shared subpopulations between tissues

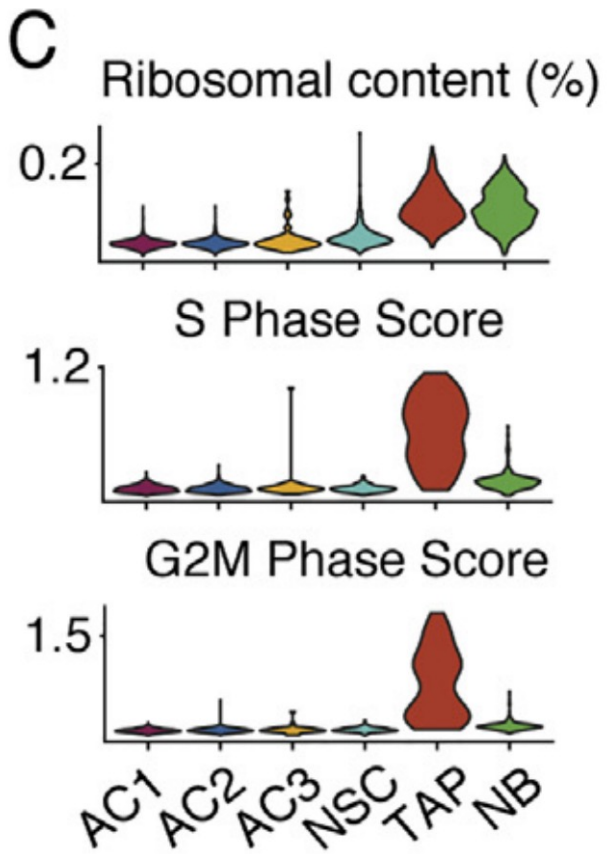
previously annotated clusters from the two datasets

newly computed clusters in the aligned space

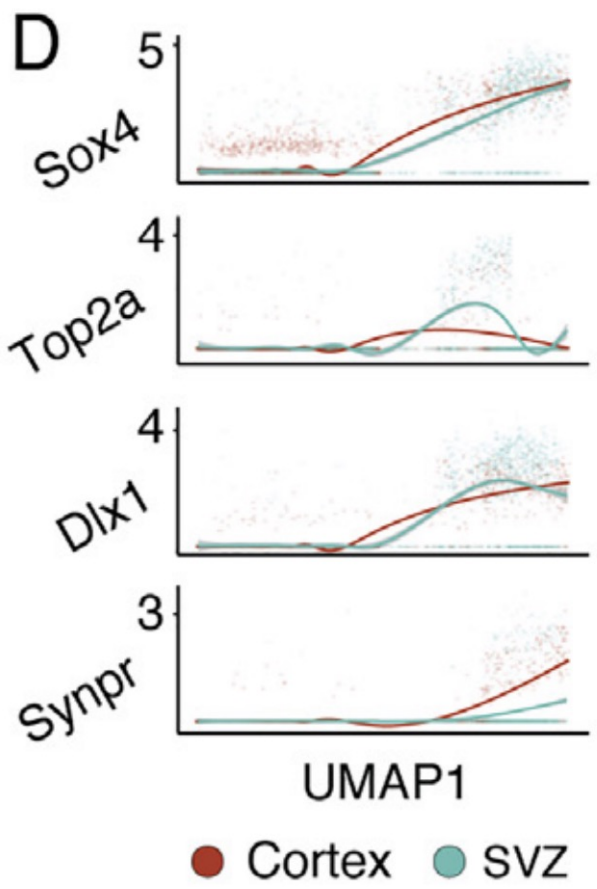


Part of AC1 and AC2 aligned to NSC, indicating molecular similarities shared between cortical astrocytes and neural stem cells

Cortical and SVZ share similar transcriptional programs driving neurogenesis



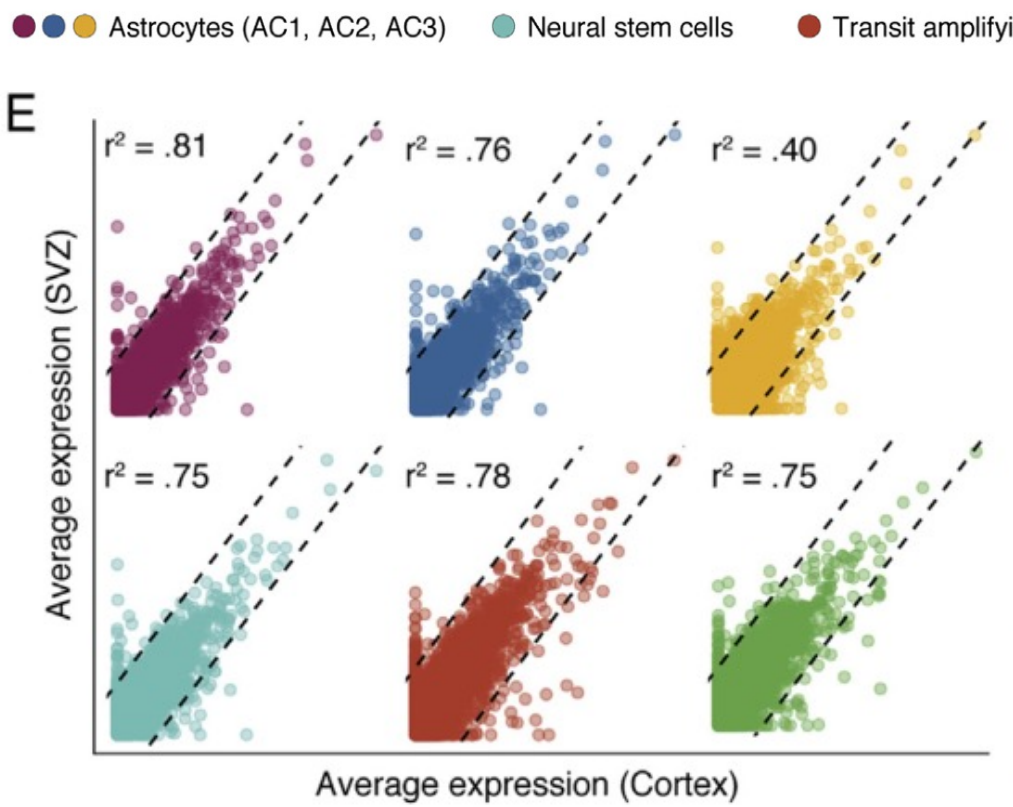
activation of ribosomal and cell cycle programs (similar to previous results of cortex neurogenic dynamical genes)

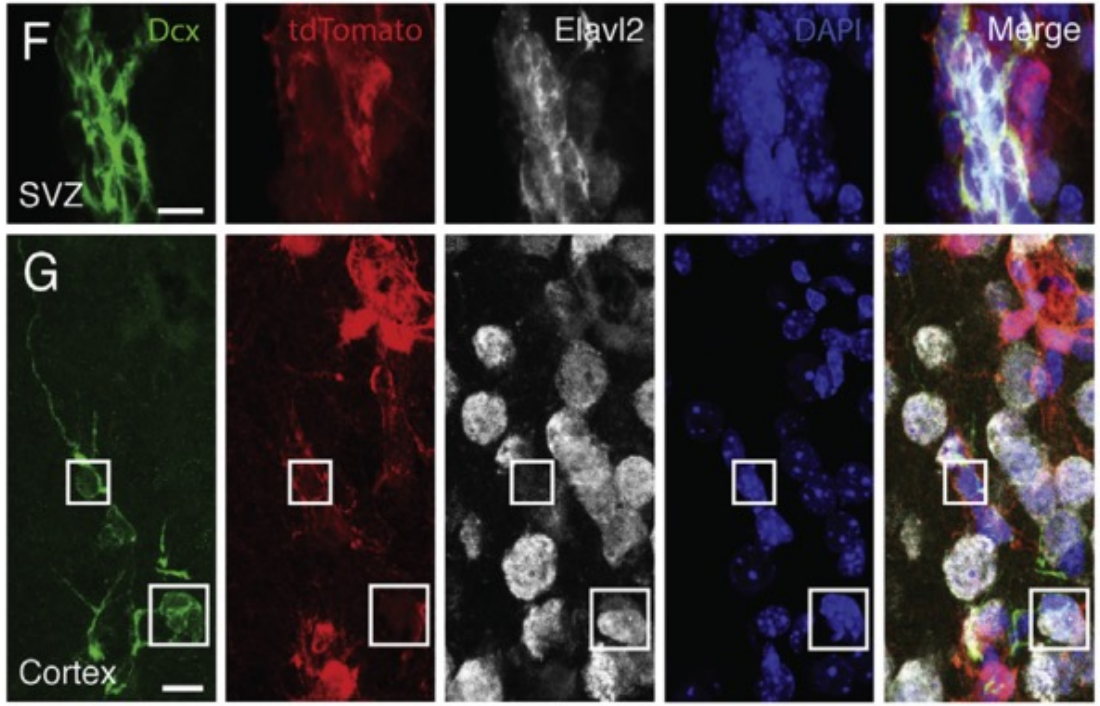


comparable gene expression dynamics of classical NSC and progenitor markers

Cortical and SVZ share similar transcriptional programs driving neurogenesis

Each point represents a gene





Highly correlated transcriptional profiles for all clusters (except AC3)

Low r^2 in AC3: likely because of the absence of brain injury and reactive gliosis in the SVZ dataset

SVZ and cortex both expressed the pan-neuronal marker Elavl2

Questions to answer

1. Previous study show that, even when Rbpj-κ is depleted from astrocytes throughout the brain, neurogenesis occurs almost exclusively in the striatum, raising questions about how widespread the neurogenic potential of astrocytes is.

2. The molecular cascade that drives the lineage fate transition is, however, unclear.

3. **It remains to be determined whether the transcriptional programs required for parenchymal astrocytes to generate adult-born neurons share similarities with canonical neurogenic processes.**

The neurogenic program cortex astrocytes use is similar to neural stem cells.

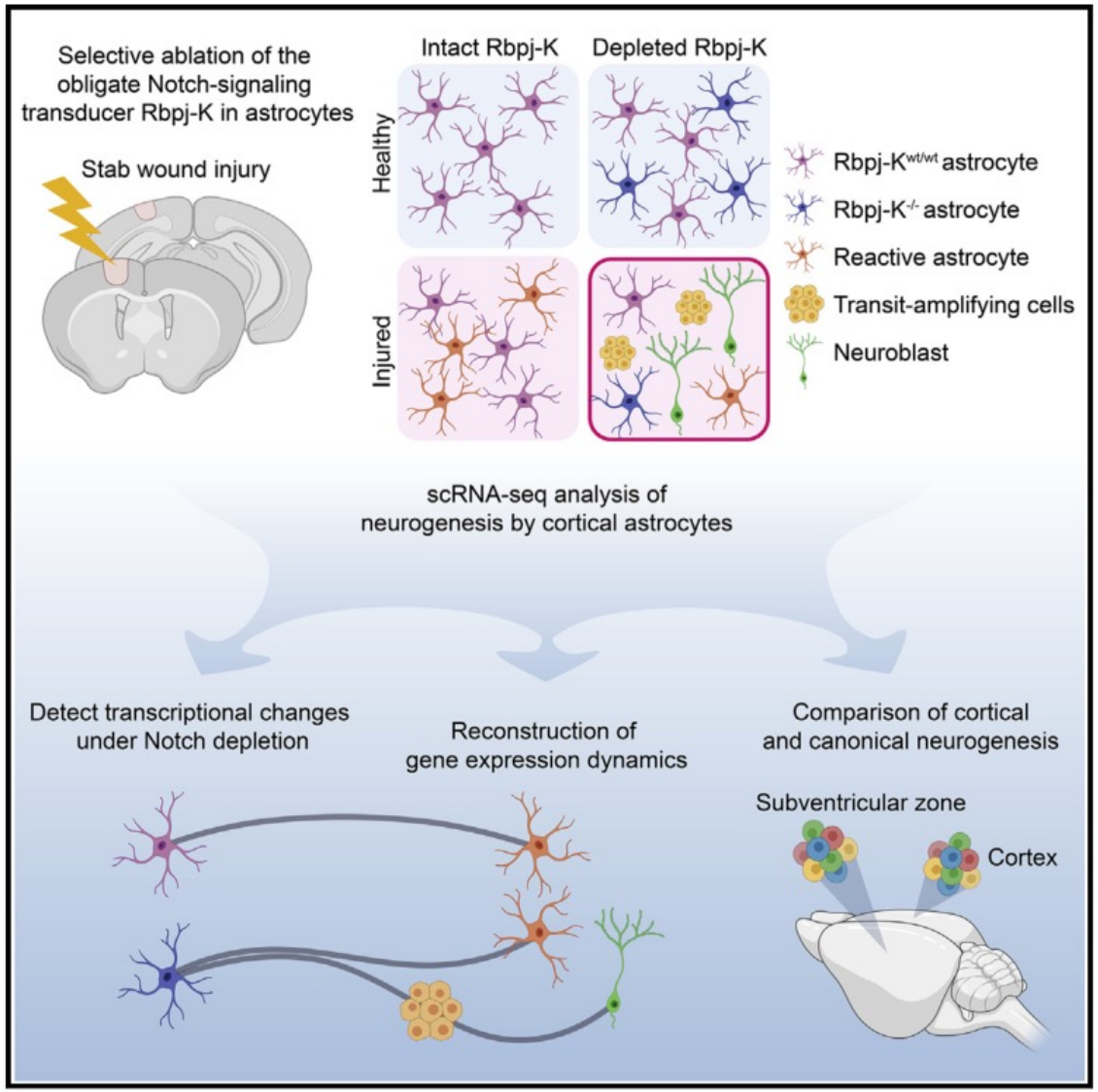
Background

1. Rbpj-κ Deletion and Stab Wound Injury Trigger Neurogenesis in the Somatosensory Cortex
2. Notch Signaling Represses a Neurogenic Transcriptional Program in Cortical Astrocytes
3. Reconstruction of Transcriptional Dynamics during Astrocyte Neurogenesis
4. Reconstruction of Gene-Regulatory Network Activity Identifies Transcriptional Programs Driving Cortical Neurogenesis
5. Cortical Neurogenesis Recapitulates Canonical Subventricular Zone Neurogenesis

Summary and discussion

Summary and discussion

Graphical Abstract



Zamboni et al. show that cortical astrocytes can generate interneurons following Notch signaling depletion and injury. Transcriptional analysis reveals early activation of a neural stem cell program that arises irrespective of injury condition. Neurogenesis by cortical astrocytes recapitulates canonical neurogenic programs and unfolds independent of reactive gliosis.

Limitations

- Other types of brain damage?
- Only a minority of Rbpj KO cells generate neuron? Additional genetic manipulation?

Thank you for your attention!