A Widespread Neurogenic Potential of Neocortical Astrocytes Is Induced by Injury

Graphical Abstract

Highlights

- Cortical astrocytes generate interneurons after Notch signaling ablation and injury
- Silencing of Notch elicits a neural stem cell program irrespective of injury
- Neurogenesis by astrocytes unfolds independent of reactive gliosis
- Cortical and subventricular zone neurogenesises display similar molecular programs

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In Brief
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.

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A Widespread Neurogenic Potential of Neocortical Astrocytes Is Induced by Injury

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SUMMARY

Parenchymal astrocytes have emerged as a potential reservoir for new neurons in non-neurogenic brain regions. It is currently unclear how astrocyte neurogenesis is controlled molecularly. Here we show that Notch signaling-deficient astrocytes can generate new neurons after injury. Using single-cell RNA sequencing, we found that, when Notch signaling is blocked, astrocytes transition to a neural stem cell-like state. However, only after injury do a few of these primed astrocytes unfold a neurogenic program, including a self-amplifying progenitor-like state. Further, reconstruction of the trajectories of individual cells allowed us to uncouple astrocyte neurogenesis from reactive gliosis, which occur along independent branches. Finally, we show that cortical neurogenesis molecularly recapitulates canonical subventricular zone neurogenesis with remarkable fidelity. Our study supports a widespread potential of parenchymal astrocytes to function as dormant neural stem cells.

INTRODUCTION

The adult mammalian brain has limited regenerative capacity after neuronal loss. Small populations of neural stem cells persist throughout life and can generate progeny that migrate to sites of damage, where they generate glia (Faiz et al., 2015) or contribute to replacement of a minor portion of neurons lost to injury (Andlsson et al., 2002). In addition to these neural stem cells, astrocytes isolated from the injured cortex display neural stem cell properties in vitro (Sirko et al., 2013). Moreover, 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. Parenchymal astrocytes thus represent a potential novel source of latent neurogenic cells, but it is unknown whether this potential is conserved throughout the brain.

In the striatum, astrocytes commit to the neuronal lineage through induction of neural stem cell-associated genes, such as Ascl1, followed by a transient phase of expansion before generating clusters of neuroblasts and, eventually, neurons (Magnusson et al., 2014). Neurogenesis by striatal astrocytes after stroke is triggered by reduced Notch signaling, and blocking Notch activity by conditional deletion of the key regulator Rbpj-k is sufficient to activate this neurogenic program, even in the absence of injury (Magnusson et al., 2014). The molecular cascade that drives the lineage fate transition is, however, unclear, and it remains to be determined whether the transcriptional programs required for parenchymal astrocytes to generate adult-born neurons share similarities with canonical neurogenic processes. Furthermore, only astrocytes in the striatum and medial cortex generate neurons when Rbpj-k is depleted, raising questions about the extent of their neurogenic potential throughout the brain.

We found that Rbpj-k-depleted astrocytes from the cortex can mount a neurogenic response, when animals are subjected to an injury. The neurogenic potential was widespread throughout cortical regions and layers because upper- and deep-layer astrocytes from the somatosensory and visual cortices initiated neurogenesis upon Rbpj-k depletion. Astrocytes close to the lesion form Ascl1-expressing transit-amplifying cells before generating proliferative clusters of double-cortin (Dcx) neuroblasts and mature neurons. Single-cell RNA sequencing (scRNA-seq) revealed that conditional knockout of Rbpj-k drives induction of a neural stem cell program in cortical astrocytes that resembles canonical neurogenic processes, as observed in transcriptome analyses of the germinai niche (Llorens-Bobadilla et al., 2015; Zywitza et al., 2018). However, only after a stab wound injury can the neurogenic program be instigated, and Notch-deficient cortical astrocytes are able to progress through amplifying cell divisions to generate GABAergic interneurons. Importantly, analyses of individual cell trajectories show that neurogenesis by astrocytes occurs independent of reactive gliosis, which is induced in parallel by a different subset of astrocytes. Altogether, our data suggest that cortical astrocytes should be viewed as a latent source of neural stem cells that can be recruited to propagate a neurogenic program with the potential to generate neurons that replace cells lost because of injury.
RESULTS

Rbpj-k Deletion and Stab Wound Injury Trigger Neurogenesis in the Somatosensory Cortex

To assess the neurogenic potential of cortical astrocytes in the injured adult brain, Cx30-CreER, Rbpj-kloxPloxP mice (Slezak et al., 2007; Tanigaki et al., 2002) on a Rosa-yellow fluorescent protein (YFP) or -tdTomato reporter background (Madisen et al., 2015; Srinivas et al., 2001) received tamoxifen to induce selective ablation of Rbpj-kloxPloxP and reporter expression in astrocytes and their progeny and were subjected to a stab wound injury (Figures 1A and 1B).

Although neurogenesis was not detected in uninjured Rbpj-kloxPloxP mice (n = 4) or after stab wound injury without homozygous deletion of Rbpj-kloxPloxP (n = 5), the combination of stimuli resulted in local parenchymal astrocytes initiating a neurogenic program (Figures 1C and 1D) through upregulation of Ascl1, followed by a transit-amplifying stage, before...
Neurogenic Cells Originate from Local Cortical Astrocytes

Neuroblasts from the subventricular zone can migrate toward cortical sites of damage, where they commit to an astroglial lineage fate (Faiz et al., 2015). Our transgenic mouse model labels not only parenchymal astrocytes but also Cx30-expressing neural stem cells in the subventricular zone (Magnusson et al., 2014). Therefore, we could not exclude that the neuroblasts observed in the cortex had migrated from the subventricular zone. To assess the origin of the neurogenic cells, we used several more restricted recombination paradigms (Figure 2).

To address whether transit-amplifying cells and neuroblasts were produced locally, we achieved selective recombination of cortical cells through focal injection of an adenovirus-associated virus carrying Cre under the Gfaa promoter in Rbpj-/- mice, and subjected the animals to stab wound injury (Figure 2A). YFP+/Dcx+ double positive cells were observed at the injury site after intra-cortical virus delivery, indicating the local cortical origin of the neural progenitors (Figures 2C–2F). Notably, the percentage of YFP+ cells expressing the neurogenic marker were comparable between local (i.e., virus-mediated, n = 7, 4.62% ± 2.54%) and systemic (i.e., tamoxifen-mediated, n = 5, 5.52% ± 4.75%) recombination (t(5.65) = –0.39, p = 0.71; Figure 2C).

We next asked whether there may be additional contributions, such as by subventricular zone migratory neuroblasts or Cx30-expressing leptomeningeal cells, and turned to focal recombination using endoxifen, an active metabolite of tamoxifen, in the same transgenic line to address this question (Figure 2B). We administered endoxifen through the lateral ventricle of Rbpj-/-mitm mice. This resulted in recombination restricted to cells at and around the subventricular zone (Figures S2E–S2G) and did not reveal migration of neuroblasts to the injured cortex (n = 6; Figure 2G).

Vascular leptomeningeal cells (VLMCs) are a population of fibroblast-like cells located at the interface between the endothelium and the astroglial endfoot layer surrounding the parenchyma and characterized by expression of Pdgfra along with collagen fiber crosslinking proteins, such as Dcn and Lum (Vanlandewijck et al., 2018; Zeisel et al., 2018). Like astrocytes, VLMCs express Cx30 (De Bock et al., 2014) and are thus recombined in our transgenic mouse line. We asked whether VLMCs may contribute to the observed cortical neurogenesis in injured Rbpj-/-mitm mice. For this purpose, we achieved selective targeting of VLMCs through intracisternal delivery of endoxifen (Zamboni et al., 2020) and subjected the mice to stab wound injury (Figures 2B and 2H–2J). The lesioned cortices of these animals were devoid of Ascl1+ or Dcx+ neural progenitors, suggesting that VLMCs do not have neurogenic potential (n = 5; Figure 2K).

To address whether the possibility to induce cortical neurogenesis is applicable to other regions of the cerebral cortex, we subjected Rbpj-/-mitm mice to stab wound injury directed to the occipital cortex (n = 4; Figures S2A–S2D). Generation of astrocyte-derived neural progenitors in the primary visual cortex (Figures S2B–S2D), a region distal from the subventricular zone, indicated that migratory neuroblasts are unlikely to contribute to the neurogenic phenomenon recorded in the cortex. It also revealed that the astroglial neurogenic potential is not restricted to astrocytes from the somatosensory cortex but extends instead over different cortical regions.

scRNA-Seq of Fate-Mapped Cortical Astrocytes and Their Progeny

Our initial investigation indicated that neurogenesis by astrocytes may proceed along developmental steps reminiscent of canonical neurogenesis (Figure S1). To investigate a more comprehensive transcriptional framework delineating the commitment of cortical astrocytes to a neuronal lineage fate, we carried out a scRNA-seq experiment. For this purpose, we dissected portions of the somatosensory cortex subjected to stab wound injury and healthy contralateral cortices of wild-type and Rbpj-/-mitm mice (Figure S3A). Using flow cytometry, we then sorted tdTomato+ cells (Figures S3B–S3D) and analyzed their transcriptome. After removal of contaminating cells (i.e., oligodendrocytes, microglia, and VLMCs), low-quality cells, and possible doublets, we retained 4,627 cells for downstream analysis (Figures S3E–S3H). We performed graph-based clustering on shared nearest neighbors and t-distributed stochastic neighbor embedding (tSNE) using the top 15 principal components and 2,512 highly variable genes as input (Table S1). Our analysis unveiled the full architecture of the data set and enabled us to detect molecular differences between wild-type and Rbpj-/-depleted cells, which clustered separately from each other (Figure S3A).
Figure 2. Neuroblasts Are Generated Locally from Astrocytes of the Somatosensory Cortex

(A and B) Experimental timelines for local recombination of cells. Intracortical (ICt) delivery of an adeno-associated virus (AAV8: Gfap-Cre) was used to demonstrate a cortical origin of the neurogenic cells (A). Delivery of endoxifen to the lateral ventricle (LV) or the cisterna magna (IC) was used to demonstrate the lack of contribution of subventricular zone and leptomeningeal cells, respectively (B). Mice were subjected to stab wound injury (SWI) 4 weeks after the local injections, and cortical tissue was collected 3 wpi for IF. See also Figure S2.

(C) The percentage of YFP+ cells expressing the neuroblast marker Dcx is comparable after ICt AAV injection and systemic tamoxifen administration ($p = 0.65$).

(E and F) Magnifications from the boxed areas in (D) display representative cluster of neuroblasts (E) and post-mitotic cell (F) labeled with YFP, demonstrating their local origin. The scale bars represent 10 μm.

(G) Tiled image illustrating the absence of Dcx+ cells around the cortical lesion site (*) following intraventricular administration of endoxifen, which indicates lack of subventricular zone contribution to the neurogenic phenomenon observed in the injured $Rbpj^{-/}$ brain. The scale bar represents 200 μm. See also Figure S2.

(H) Tiled image of one hemisphere from a Cx30-tdTomato mouse after intracisternal delivery of endoxifen. Vascular leptomeningeal cells (VLMCs) surrounding the cortex and penetrating the parenchyma alongside blood vessels become recombined with this injection. The scale bar represents 500 μm.

(I and J) Magnifications of the boxed areas in (H), displaying examples of perivascular leptomeningeal cells in the lateral (I) and medial (J) cortex. The scale bars represent 200 μm.

(K) Tiled image illustrating the absence of Ascl1- or Dcx-expressing cells in injured Cx30-CreER; $Rbpj^{-/}$ mice following intracisternal delivery of endoxifen, which indicates that VLMCs are not a source of neurogenic cells. The scale bar represents 150 μm.
Notch Signaling Represses a Neurogenic Transcriptional Program in Cortical Astrocytes

Transit-amplifying cells and neuroblasts from injured cortices of Rbpj-κ^fl/fl mice segregated away from astrocytes (Figure 3A), based on the expression of genes implicated in the cell cycle (e.g., Mki67, Ccnd2, and Ptma) and neuronal specification (e.g., Dcx, Sp9, and Igfbp1; Figures 3B, 3C, and 3E; Table S2). Astrocyte cluster 1 (AC1, n = 1,978) was composed of cells enriched in genes such as Hes5, Ubc, and Nrarp, indicative of active Notch signaling (Figure 3B and 3C; Table S2). This cluster was composed of astrocytes with intact Rbpj-κ as well as some astroglia from animals carrying the Rbpj-κ null allele.

Figure 3. Notch Represses a Neurogenic Transcriptional Program in Cortical Astrocytes

(A) The tSNE plot distributes cells based on previously computed highly variable genes organized in the top 15 principal components (see also Table S1 and Figure S3). Unsupervised clustering reveals the presence of three astrocyte clusters (AC1–AC3) that segregate from the neurogenic progeny (neuroblasts [NBs]). Reported in the legend is the total number of cells assigned to each cluster.

(B) The heatmap represents the average enrichment of the top 50 differentially expressed genes for each group. On the right is a list of representative cluster markers ordered according to their fold change. See also Table S2.

(C) Violin plots reporting expression levels across clusters for a few representative genes.

(D) Barplot reporting the distribution of samples (i.e., Rbpjk^-κ^fl/fl + injury, Rbpjk^-κ^WT/WT + healthy, Rbpjk^-κ^WT/WT + injury, and Rbpjk^-κ^WT/WT + healthy) across clusters. Approximately half of the astrocytes sampled from Rbpj^-κ^fl/fl mice are assigned to AC2 regardless of injury condition, whereas Rbpj^-κ^WT/WT glia are predominantly found in AC1. Neurogenic cells from NBs primarily originated from the Rbpj^-κ^fl/fl + injury condition (n = 329), although a few NBs could be found under control conditions (n = 3), likely because of unintended inclusion of contaminating cells from the rostral migratory stream.

(E) Enrichment of representative genes expressed in wild-type astrocytes (Hes5), neurogenic glia (Ascl1), transit-amplifying cells (Mki67), and NBs (Dcx). See also Tables S2 and S3.

(F) Astrocytes expressing genes that identify surface (Gfap and Id3), upper-layer (Chrdl1), and deep-layer (Il33 and Id3) glia can be found as AC1 and AC2, indicating that the neurogenic potential is conserved across astroglial subtypes.
AC2 expressed the anti-proliferative gene Btg2 shown at early stages in the neurogenic program (Fior and Henrique, 2005; Matsunaga et al., 2015). Additionally, glia in Figure 3B; Table S3), whose induction has been sis during central nervous system development (Iacopetti for neuron-generating progenitors at the onset of neurogene-
et al., 2015; Wilkinson et al., 2013). AC2 cells were enriched in genes that are direct targets of Ascl1 in genes implicated in immune response, such as Ifitm3 and C4b (Figures 3B and 3C; Table S2). Notably, AC3 cells displayed activation of genes that are characteristic of type A1 reactive glia (i.e., Cxcl10, H2-T23, Il1p1, and Psmb9; Figure 3B), identified by Liddelow et al. (2017).

We next performed differential expression analysis to further investigate the transcriptional differences between AC1 and AC2 (Table S3). Rbpj-κ depletion in AC2 induced activation of transcription factors such as Ascl1, Neurog1, and Olig2 (Figures 3B and 3C), which promote neuronal specification during corticogenesis in the developing brain (Liu et al., 2015; Wilkinson et al., 2013). AC2 cells were enriched in genes that are direct targets of Ascl1, such as Hes6 and Gadd45g (Figure 3B; Table S3), whose induction has been shown at early stages in the neurogenic program (Fior and Henrique, 2005; Matsunaga et al., 2015). Additionally, glia in AC2 expressed the anti-proliferative gene Btg2 (also known as Tis21; Figure 3C), which has been recognized as a marker for neuron-generating progenitors at the onset of neurogenesis during central nervous system development (iacopetti et al., 1999). Similar to other studies (Attardo et al., 2008, 2010) reporting its expression throughout the neurogenic program, Btg2 could be found in our dataset in AC2 and the neural progenitor cluster (Figure 3C). Moreover, conditional deletion of Rbpj-κ led to downregulation of genes that are typically associated with neural stem cell quiescence in the adult neurogenic niches (Figure 3B; Table S3). Among these, Hes5, Hey1, Hes1, and Id4 are transcription factors that exert negative regulation of neurogenesis. Notably, compared with AC2, AC1 was enriched in genes implicated in cholesterol biosynthesis (e.g., Gene Ontology term GO:006695, Cyp4f15, Fdps, and Hmgcs1; Figure 3B), a key function astrocytes undertake to sustain the brain’s metabolic requirements under physiological conditions (Ferris et al., 2017).

Recent studies have identified transcriptional differences between subtypes of cortical astrocytes (Bayraktar et al., 2020; Lanjakornsiripan et al., 2018; Zeisel et al., 2018). Although our scRNA-seq dataset was not able to segregate astrocytes based on their origin within the cortical layers, we could observe cells enriched in surface (Glap and Idd3), upper-layer (Chrd1), and deep-layer (Ild3 and Idd3) markers in the wild-type (i.e., AC1) and neurogenic (i.e., AC2) astrocyte clusters (Figure 3F). This indicated that initiation of the neurogenic program is not restricted to a subtype of cortical astrocytes but is widespread throughout the cortical layers.

Taken together, our scRNA-seq analysis enabled us to distinguish clusters of cortical astrocytes and neural progenitors that originated from local astroglia. Furthermore, differential expression analysis revealed that AC2 cells downregulate genes related to astrocyte-specific metabolic functions while concomitantly acquiring a transcriptional profile reminiscent of primed but still quiescent neural stem cells.

Reconstruction of Transcriptional Dynamics during Astrocyte Neurogenesis

We performed pseudotemporal analysis to computationally resolve the neurogenic trajectory and capture the molecular dynamics that drive the lineage fate conversion from astrocyte to neuron (Figure 4). For this purpose, we ran diffusion map (destiny v.2.12), a non-linear dimensionality reduction approach that reconstructs dynamic transcriptional programs and orders data points along specific differentiation traces (Angerer et al., 2016). Gene loadings from previously computed principal-component analyses were provided as input (Table S1) after removing transcripts implicated in the cell cycle, which would otherwise dominate and skew the resulting pseudotemporal trajectory. Visualization of the top five dimensions calculated with a diffusion map allowed us to draw a continuum that connected astrocytes to their neurogenic progeny (Figure 4A and S4A). Astrocytes with intact Notch signaling (AC1) were polarized at one end of the pseudotemporal scale. Conversely, some astrocytes entering the neurogenic program (AC2) progressed toward a neural progenitor state, spreading along the x axis. The compactness of astrocytes relative to the neuroblast cluster on the diffusion map likely reflected the increased transcriptional heterogeneity across states as cells transition toward the neuronal lineage (Figure 4A). Notably, the diffusion map revealed that AC3 reactive astrocytes formed a separate branch that did not feed into the main neurogenic path (Figure 4A). Accordingly, these astrocytes rarely expressed the proneural transcription factor Ascl1 (expressed at low levels in 8% of these cells compared with 38% of cells in AC2; Figures 3C, 4B, and 4C). Instead, they were highly enriched in inflammatory response-related genes (Figure 3B; Table S2) and displayed hypertrophic processes, indicative of a reactive glia phenotype (Figure 4B).

We assigned a pseudotemporal score to cells ordered along the first diffusion map component, which recapitulated the neurogenic trajectory (Figure 4A), and used this to detect transcriptional changes driving the lineage fate transition. Differential expression analysis across pseudotime was performed with Monocle v.2.10 (Qiu et al., 2017) and revealed the presence of five transcriptional signatures that identify distinct cell states along the neurogenic path (Figure 4D; Table S4). Gene Ontology analysis performed on each gene expression module enabled us to gain functional insight into the transcriptional programs characterizing each state (Figure 4D; Table S4). Modules 1 and 2 encompassed genes activated during the initial pseudotemporal phases. These were functionally related to the astroglial physiological roles of lipid (e.g., S1c27a1 and Pcyt2) and carbohydrate (e.g., Chst1) biosynthesis along with glutamate (e.g., Slc1a2 and Slc1a3) and water (e.g., Aqp4) transport. Downregulation of these transcripts was followed by a surge in the expression of ribosomal genes (e.g., Rpsl, module 3; Figures 4D and 4E), which reflects the need for neurogenic cells to initiate protein biosynthesis programs in preparation for the lineage transition (Lorens-Bobadilla et al., 2015). Of note, higher ribosomal content was already displayed...
Figure 4. Pseudotemporal Reconstruction of the Neurogenic Trajectory Undertaken by Cortical Astrocytes following Rbpj-κ Silencing and SWI

(A) The diffusion map overlaid onto a shared nearest neighbors graph connects transcriptionally similar cells while recapitulating the neurogenic trajectory and segregating reactive astrocytes on a separate trace. Astrocyte cluster 1 (AC1) cells are polarized toward one end of the continuum, whereas AC2 feeds into the NB fate, which progresses along the first diffusion map component (DM1). Cells from AC3 branch out to form a separated trajectory. See also Figure S4.

(B) Pictures of injured cortices of Cx30-Rbpj-κ/fl/fl-tdTomato mice, validating that Stat1+ astrocytes present hypertrophic morphologies typical of reactive glia and do not express Ascl1. In contrast, Ascl1+ astrocytes do not display hallmarks of gliosis. The scale bars represent 20 μm.

(C) Gene expression levels demonstrate that AC3 cells are mostly devoid of Ascl1 transcripts but enriched in genes implicated in reactive gliosis, such as Stat1 and Iigp1.

(D) Heatmap reporting the top 1,000 dynamically expressed genes across DM1 along with a few representative Gene Ontology terms and transcripts enriched in each module. Genes are categorized into five transcriptional signatures and enriched at different stages along the neurogenic trajectory. See also Table S4.

(E) Violin plots indicating the per-group normalized average score for ribosomal activity and the cell cycle. Induction of cell cycle genes is unique to the NB cluster, unlike ribosomal genes, which are already activated in a small portion of astrocytes from AC2.

(F) Gene expression levels plotted along the diffusion map indicate that astrocyte-generated NBs are activating genes implicated in a GABAergic interneuronal fate, which are enriched since early proliferative phases (i.e., Dlx1 and Dlx2), or specific to a more mature phenotype (i.e., Htr3a and Synpr). See also Table S2.
in a small portion of astrocytes from AC2 (Figure 4E), denoting their early commitment to the lineage fate switch. Concomitantly, cells induced expression of genes implicated in chromatin modification (e.g., Hdac2) as well as genes implicated in neurodevelopment (e.g., Dlx1, Dlx2, and Sox4; module 3). Module 4 was dominated by transient expression of cell cycle genes (e.g., Aurkb, Top2a, and Pcnr; Figures 4D and 4E) and was followed by induction of transcripts, clustered under module 5, related to neuronal fate specification (e.g., Dcx, Dlx5, and Stmn4) and axonogenesis (e.g., Ncam1, Cd24a, and Klf7). Post-mitotic neuroblasts located at the end of the neurogenic continuum specifically expressed transcription factors (e.g., Dlx5, Dlx6, and Sp9) indicative of a GABAergic interneuronal phenotype (Figure 4F). At the latest maturation stages, they also upregulated key GABAergic signaling components (e.g., Gad1/2 and Htr3a) and genes implicated in synapse formation and maintenance (e.g., Synpr, Syn1, and Snap25; Figure 4F).

Altogether, our pseudotemporal analysis provided functional insight into the transcriptional changes driving lineage fate transition and revealed that cortical astrocytes subjected to Rbpj-κ deletion and stab wound injury are primed to generate GABAergic interneurons.

Reconstruction of Gene-Regulatory Network Activity Identifies Transcriptional Programs Driving Cortical Neurogenesis

We next aimed to determine sets of transcription factors and target genes that are dynamically activated throughout the neurogenic process. For this purpose, we first subclustered AC2 based on pseudotemporal ordering (i.e., assigning cells to a new cluster, AC4, whenever their pseudotime score exceeded the maximum score reached by AC1 astrocytes). This cluster identified a small group of cells captured at the time of transition to the neuronal lineage (Figure 5A). We then utilized SCENIC (single-cell regulatory network inference and clustering) for identification and characterization of active gene-regulatory networks across the dataset (Abar et al., 2017). The workflow produced a list of 206 regulons (i.e., transcription factors and their direct targets) that showed enriched motifs for the corresponding transcription factor (Table S5). We next scored regulon activity for each cell in the data using AUCell (v1.4.1) and computed the percentages of cells in each group with an active regulon (Figure 5B). Our analysis identified astroglial and neuronal regulons, active in AC1 to AC4, and neuroblast clusters, respectively. Notably, the AC4 transcriptional profile appeared to lie at the interface between astroglial and neuronal identities (Figure 5B). Indeed, the activity of regulons highly expressed in AC1–AC3 (e.g., Esr1 and Olig2) was decreased in AC4, whereas neuron-specific transcriptional programs (e.g., Dlx1 and Sp9) started to be induced in this cluster even before cell division (Figures 5C and 5D). SCENIC also detected transcriptional programs enriched in AC3 reactive glia, which showed activation of transcription factors, such as Stat1 and Stat2, but not of neurogenesis-related regulons (Figures 5B–5D; Table S5).

Overall, gene-regulatory network analysis allowed us to reveal early activation of neurogenic transcriptional programs prior to cell division and neuronal fate specification, indicative of early neurogenic priming in astrocytes.

Combined Transcriptional Analysis of Cortical and Subventricular Zone Neurogenesis Reveals Convergent Neurogenic Programs

Pseudotemporal analysis of cortical neurogenesis revealed that combined Rbpj-κ deletion and stab wound injury elicit a molecular program similar to that implicated in canonical neurogenesis. To make a direct comparison between transcriptional changes underlying neural fate commitment in the cortex and the germinal niche, we aligned our dataset to a published scRNA-seq experiment (Zywitzka et al., 2018) encompassing subventricular zone cells (Figure S5) using canonical correlation analysis (Butler et al., 2018). The subventricular zone dataset was first cleared of cells not implicated in neurogenesis (Figure S5). Filtered data were subsequently processed alongside our dataset to identify shared subpopulations between tissues (Figure 6A). Following dataset alignment, we re-ran the clustering algorithm and noticed that the integrated data maintained a similar partition among astrocyte subgroups and clusters of quiescent neuronal stem cells, cycling cells, and neuroblasts, as projected by UMAP (Uniform Manifold Approximation and Projection) visualizations (Figure 6A) and identification of cluster biomarkers (Figure 6B; Table S6). Niche astrocytes distributed relatively uniformly across the astrocyte groups, with a slight preference for AC1, whereas subventricular zone stem cells were predominantly assigned to the neural stem cell (NSC) cluster identified in the integrated dataset (Figure S6A). A few cortical astrocytes from each experimental condition clustered under the NSC group in the aligned space (Figure S6B), likely because of the molecular similarities shared between astrocytes and quiescent stem cells (Figures 6B and S6C). Nevertheless, subventricular zone cells within the cluster were enriched in canonical NSC transcripts, such as Thbs4 and Rlaid1, compared with cortical astrocytes grouping together (Figure S6D).

Integration of the datasets confirmed a remarkable similarity in the transcriptional programs driving cortical and subventricular zone neurogenesis, as suggested by activation of ribosomal and cell cycle programs, and comparable gene expression dynamics of classical NSC and progenitor markers (Figures 6C, 6D, 6E, and S6F). We furthermore computed group-wise gene expression averages for both datasets and found a high correlation ($r^2 > 0.75$) between transcriptional profiles for all clusters except AC3 ($r^2 = 0.4$), likely because of the absence of brain injury and reactive gliosis in the niche dataset (Figure 6E). Few genes appeared to be differentially expressed between regions (e.g., Id4 and Igfbp2 enriched in the subventricular zone and cortex, respectively; Figures S6G and S6H), and in both areas, neuroblasts displayed GABAergic identities (Table S6) and expressed the pan-neuronal marker Elavl2, which characterizes neuron-committed progenitors and mature cells (Figures 6F and 6G).

Alignment of the transcriptional events driving neuronal specification in the cortex and the neurogenic niche further supports the idea that parenchymal astrocytes may be viewed as dormant NSCs that, with the appropriate molecular cues, can be recruited to generate neurons following a trajectory similar to canonical neurogenesis.

**DISCUSSION**

Striatal astrocytes are capable of generating neurons in mice when subjected to injuries such as stroke or excitotoxic damage.
Magnusson et al., 2014; Nato et al., 2015). This process is initiated by Notch inhibition. Interestingly, 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. The present study revealed that astrocytes throughout the layers of the somatosensory and visual cortices can be recruited into a neurogenic program upon Notch signaling inhibition when mice undergo a stab wound injury. Furthermore, scRNA-seq analysis allowed us to reconstruct the full molecular trajectory and unveil a comprehensive transcriptional framework central to the astrocyte-to-neuron fate switch.

Notch signaling depletion is crucial for instructing initiation of the neurogenic phase from radial glia during development (Bansod et al., 2017). Notch controls expression of the basic-helix-loop-helix transcription factors Hes1 and Hes5, which, in turn, act as negative regulators of the pro-neuronal genes Ascl1, Neurog1, and Neurog2 (Hatakeyama et al., 2004; Kageyama et al., 2007). We showed that, similar to NSCs, Notch depletion in cortical astrocytes leads to downregulation of Hes5 and consequent expression of Ascl1 along with its downstream targets (e.g., Hes6 and Gadd45 g). Interestingly, we recorded increased expression of NSC-related genes in Rbpj-κ-depleted astrocytes from healthy and injured cortices. However, only when animals are subjected to injury do glia progress along the neurogenic path to generate neurons. Of note, other studies have reported increased reprogramming efficiency in the presence of cortical damage (Buffo et al., 2008; Guo et al., 2014; Sirko et al., 2013), although our transcriptional data suggest that astrocytes do not need to display signs of reactive gliosis to enter the neurogenic program. Rbpj-κ-depleted astrocytes activate transcriptional signatures of NSCs that make them more amenable to lineage progression toward a neuronal fate. In addition, the cortical microenvironment following injury may change from a highly gliogenic region to support neuronal specification. Similar to the subventricular zone, with its privileged access to the vascular compartment (Tavazoie et al., 2008) and cerebrospinal fluid (Silva-Vargas et al., 2016), pervasive injury to the cortex may lead to an increased neurogenic potential in astrocytes.
allow extravasation of growth factors in the parenchyma, which may support neurogenesis. Sirko et al. (2013) have shown that sonic hedgehog, supplied by blood and cerebrospinal fluid, modulates proliferation of reactive astrocytes and neurosphere formation. Additionally, cerebrospinal fluid-borne factors, such as BMP5 and IGF1, regulate stem cell activity in the subventricular zone (Silva-Vargas et al., 2016) and may promote neurogenesis in the cortex following injury.

Several reprogramming studies have generated neurons through overexpression of master regulators of neurogenesis, including Ascl1, NeuroD1, and Ngn2 (Gascón et al., 2016; Guo et al., 2014; Liu et al., 2015). In contrast to our findings, however, these manipulations do not lead glia through a transit-amplifying phase before committing to the neuronal lineage. According to our data, each Rbpj-κ-deleted astrocyte from the injured cortex can give rise to up to 40 neuroblasts through cell division, which

Figure 6. Cortical Neurogenesis Recapitulates Canonical Subventricular Zone Neurogenesis
(A) UMAP plot displaying subventricular zone data (Zywitza et al., 2018) aligned to the current cortical dataset. The integrated dataset maintains partitions between astrocyte clusters (AC1–AC3) as seen for the cortex and identifies clusters of neural stem cells (NSCs), transit-amplifying cells (TAPs), and NBs. See also Figures S5 and S6.
(B) Heatmap displaying the top 50 enriched transcripts for each cluster and reporting a few examples. See also Table S6.
(C) Violin plots showing changes in expression of ribosomal genes (reported as a percentage on the total number of genes) and cell cycle genes (reported as normalized S and G2M phase scores) across clusters.
(D) Plots highlighting the dynamic induction of classic neural progenitor markers between the cortical and subventricular zone datasets. The first UMAP dimension was used on the x axis because it recapitulates the neurogenic trajectory. See also Figure S6.
(E) The average expression of each gene detected in the integrated dataset is compared between cortical and subventricular zone cells to reveal highly correlating transcriptional profiles ($r^2 \geq 0.75$) for all clusters except AC3 ($r^2 > 0.40$). A dashed line indicates a 2-fold change in gene expression level.
(F and G) The neuronal marker Elavl2 could be detected in Dcx+ NBs from the subventricular zone (F) and the cortex (G). Scale bars represent 20 μm.
may enable replacement of neuronal cells while preventing local depletion of glia.

Recent studies reconstructing the lineage transition of direct neuronal reprogramming through transcriptomics analysis have reported emergence of aberrant lineage choices that separate from the neurogenic trajectory at intermediate stages of transdifferentiation (Karow et al., 2018; Treutlein et al., 2016). The authors attributed commitment of the reprogrammed cells to the alternative myogenic lineage to failure to induce pro-neuronal transcriptional programs that lead to upregulation of the synapse-related genes Stmn3, Snap25, and Gria2 (Treutlein et al., 2016). Using scRNA-seq, we were able to provide a comprehensive framework of molecular changes driving astrocyte-to-neuron transition, from early activation of a neurogenic transcriptional network to expansion of progenitors and finally commitment to a GABAergic lineage fate. The newly generated neurons developed complex interneuron-like morphologies and expressed genes implicated in synapse formation and GABAergic signaling. We did not observe induction of alternative fates and were able to confirm that cortical neurogenesis recapitulates canonical neurogenic programs with remarkable fidelity. Further investigation of transcriptional networks implicated in the lineage fate switch could reveal novel regulators of neurogenesis. Thus, to aid discovery of pro-neurogenic factors, we made our data publicly available (GEO: GSE139842) and organized the results in a searchable database that allows interrogation of expression profiles of the desired genes (https://cortical-neurogenesis.shinyapps.io/cortical-neurogenesis/).

It has been hypothesized that parenchymal astrocytes may be considered dormant NSCs because of shared expression of several biomarkers. The present study further supports this notion by showing that, when recruited into the neurogenic program, the transcriptional behavior of committed glia parallels that of adult NSCs from the germinial niche.

Limitations of Study
The present study showed that neurogenesis by cortical astrocytes occurs after Notch signaling depletion in the context of traumatic injury (i.e., stab wound injury). It remains to be determined whether astrocytes may be recruited into the neurogenic program following other types of brain damage, such as neurodegenerative disease.

We recorded activation of an NSC program in a large number of Rbpj-/-depleted astrocytes; however, only a minority of these appear to undertake the neurogenic path to give rise to neuroblasts and, eventually, mature neurons. Future studies could combine Rbpj silencing with additional genetic manipulation to enhance neurogenic output. Our transcriptional analysis and searchable database may be utilized for discovery of genes that are implicated in initiating neurogenesis by astrocytes and promote survival of neural progenitors throughout the maturation process.

STAR Methods
Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.stem.2020.07.006.

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AUTHOR CONTRIBUTIONS
Conceptualization, M.Z., E.L.-B., J.P.M., and J.F.; Methodology, M.Z. and E.L.-B.; Software, M.Z.; Formal analysis, M.Z.; Investigation, M.Z. and E.L.-B.; Data Curation, M.Z.; Writing – Original Draft, M.Z.; Writing – Review & Editing, M.Z., E.L.-B., J.P.M., and J.F.; Visualization, M.Z. and E.L.-B.; Funding Acquisition, J.F.; Supervision, J.F.

DECLARATION OF INTERESTS
E.L.-B. and J.F. are consultants to 10X Genomics.

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### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| rabbit anti-Ascl1 (1:500) | CosmoBio | AB_10709354 |
| goat anti-Dcx (1:500)   | Santa Cruz | AB_2088491 |
| rabbit anti-Elavl2 (1:200) | Atlas | AB_2684918 |
| rabbit anti-Gfap (1:1000) | Dako | AB_2811722 |
| chicken anti-GFP (1:2000) | Aves Labs | AB_2734732 |
| rat anti-Ki67 (1:500)   | eBioscience | Cat# 14-5698-80 |
| mouse anti-NeuN (1:500) | Merck | AB_2298772 |
| rabbit anti-S100 (1:1000) | Dako | AB_2811056 |
| mouse anti-Stat1 (1:200) | Abcam | AB_882708 |
| Cd45-APC/Cy7          | BD Biosciences | AB_396774 |
| Ter119-APC/Cy7        | AB_2137788 |
| Bacterial and Virus Strains |        |            |
| AAV8:Gfap(2.2)-Cre    | Vector Biolabs | VB1172 |
| Chemicals, Peptides, and Recombinant Proteins |        |            |
| Dulbecco’s PBS        | Sigma | Cat# D4031 |
| Tamoxifen             | Sigma | Cat# T5648 |
| Endoxifen             | Sigma | Cat# SML2368 |
| EdU                   | Thermo Fisher | Cat# E10415 |
| Critical Commercial Assays |        |            |
| Click-iT EdU Cell Proliferation kit for Imaging | Thermo Fisher | Cat# 10340 |
| Adult Brain Dissociation kit | Miltenyi Biotec | Cat# 130-107-677 |
| Single Cell 3’ Library & Gel Bead kit | 10X Genomics | Cat# PN-120237 |
| Deposited Data        |        |            |
| scRNaseq (raw data and gene expression matrices) | This paper | GEO: GSE139842 |
| Experimental Models: Organisms/Strains |        |            |
| Cx30-CreER; Rbpj/-kfl/fl, Rbpj/-kfl/wt, or Rbpj/-kwt/wt; R26-YFP or -tdTomato | This paper | N/A |
| Software and Algorithms |        |            |
| Code for scRNaseq analysis | [https://github.com/marzamKI/](https://github.com/marzamKI/neurogenic_astros) | N/A |
| ImageJ                | [https://imagej.nih.gov/ij/](https://imagej.nih.gov/ij/) | 1.52r |
| NeuronJ               | Meijering et al. (2004) | 1.4.3 |
| cellranger            | 10X Genomics | 2.0.1 |
| R                     | CRAN | 3.6.1 |
| Seurat                | Butler et al. (2018) | 2.3.4 |
| destiny               | Angerer et al. (2016) | 2.12.0 |
| monocle               | Qiu et al. (2017) | 2.8.0 |
| ggplot2               | Wickham (2016) | 3.0.0 |
| heatmap               | [https://github.com/raivokolde/heatmap](https://github.com/raivokolde/heatmap) | 1.0.12 |
| alluvial              | [https://github.com/mbojan/alluvial](https://github.com/mbojan/alluvial) | 0.1-2 |
| GENIE3                | Albar et al. (2017) | 1.4.3 |
| RcisTarget            | Albar et al. (2017) | 1.2.1 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead Contact
Communicate with the Lead Contact, Jonas Frisén (jonas.frisen@ki.se) to obtain further details and request resources and materials used in the present study.

Materials Availability
Mouse lines and materials used in this study are available upon request.

Data and Code Availability
Raw data and gene expression matrices have been deposited at GEO under the accession code GEO: GSE139842. Code used to analyze the transcriptional data is available on github (https://github.com/marzamKI/neurogenic_astros) and a searchable database to inspect gene expression profiles for cortical and niche neurogenesis can be found at https://cortical-neurogenesis.shinyapps.io/cortical-neurogenesis/.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice
Experimental procedures were carried out in compliance with governmental and ethical specifications provided by the institution (Karolinska Institute, Sweden) and approved by the Stockholms Norra Djurförsökssetiska Nämnd. Mice were maintained on a 12 h-light/dark cycle with water and food available ad libitum.

We used Connexin-30 (Cx30)-CreER mice (Slezak et al., 2007) carrying Rbpj−/−, Rbpj−/+ or Rbpj−/+ genes (Tanigaki et al., 2002), and either R26R-YFP (Srinivas et al., 2001) or R26R-tdTomato (Madisen et al., 2015) for genetic fate mapping. Transgenic mice used in this study were on a C57BL/6j background. The animals were > 2 months old and weighted 25-35 g when entering experiments.

For the immunofluorescence experiments both male and female mice where utilized, whereas only females were used for the scRNaseq experiments. Littermates were randomly assigned to each experimental condition, and investigators were blinded to the allocation during quantifications and assessment of the outcomes resulting from genetic and surgical manipulations.

METHOD DETAILS

Sample size
Each immunofluorescence experiment utilized 4 to 7 mice per experimental condition. The sample size required for the scRNaseq experiment was calculated based on the yield of viable cells obtained during pilot experiments (data not shown). Additionally, since abundance of neurogenic cells occurring in the Rbpj−/−− Injury condition was expected to be lower than that of astrocytes (5%–15% of tdTomato+ cells), we collected a larger number of cells for this sample, thus ensuring a good representation of the rarest population.

Induction of the conditional knockout
Genetic recombination of astrocytes throughout the parenchyma was induced upon systemic exposure to tamoxifen (Sigma; 20 mg/ml in corn oil with 10% ethanol), administered daily for five consecutive days (oral gavage, 2 mg/ml). Local recombination of cortical astrocytes was performed with a single intracranial injection of an adenov-associated virus (serotype 8) carrying Cre under a Gfap(2.2) promoter (VB1172, Vector Biolabs), whereas leptomeningeal cells and subventricular zone cells were recombined through intracisternal or intraventricular injections of endoxifen, respectively (Sigma, 0.5 mg/ml in 5% DMSO; see Drug administration protocols). Time points for each experiment were counted from the last day of gavage/injection.
Drug administration protocols
Surgical procedures were carried out four weeks after tamoxifen administration, on mice kept under general anesthesia (4% isoflurane for induction, and 1.5%–2.5% isoflurane for maintenance) and fixed onto a stereotactic frame. For local recombination of cortical astrocytes, 0.5 μL of Gfap-Cre adenovector (≥ 10^12 GC) was injected using a 36G blunt needle (World Precision Instruments, WPI) mounted on a micromanipulator, and infused at 100 nl/min (+0.5 mm antero-posterior, +2.5 mm medio-lateral from Bregma, and –0.5 mm deep from dura). For recombination of leptomeningeal, endoxifen (10 μl, 0.5 mg/ml) was injected manually into the cisterna magna using Hamilton syringes with a 30G beveled needle bent to 30° at 3 mm from the tip (Zamboni et al., 2020). Finally, recombination of cells at, and around, the subventricular zone was achieved through intraventricular injection of endoxifen (2 μl, 0.5 mg/ml) at –0.2 mm antero-posterior, +1 mm medio-lateral from Bregma, and –1.75 mm deep from dura, and delivered using a 33G beveled needle (World Precision Instruments, WPI) mounted onto a micromanipulator. Delivery rate was set at 500 nl/min.

EdU (0.2 mg/ml) was added to the drinking water and administered throughout the experiment, starting immediately after subjecting the animals to the injury protocol.

Injury modeling
A unilateral stab wound injury was performed on anesthetized animals using a WPI syringe with a 26G blunt needle inserted in the cortical parenchyma. For injury directed to the somatosensory cortex, the needle was inserted at +0.5 mm antero-posterior, +2.5 mm medio-lateral from Bregma, and –0.5 mm deep from dura. For injury to the occipital cortex, the coordinates used were: –3.0 mm antero-posterior, +2.5 mm medio-lateral from Bregma, and –0.5 mm deep from dura. To extend the lesioned area, the needle was moved ~1 mm back and forth along the rostral-caudal axis using a stereotactic apparatus.

Immunohistochemistry
Mice were euthanized with sodium pentobarbital (100 μl/intraperitoneal injection) and transcardially perfused with ice-cold PBS followed by 4% formaldehyde. After overnight fixation in 4% formaldehyde at 4°C, brains were vibratome-sectioned into 50 μm-thick coronal slices. Tissue sections were first treated with blocking buffer (10% donkey serum, 0.3% Triton X-100, and 0.04% NaN3 in PBS; 1 h at room temperature), followed by primary (overnight at 4°C) and secondary antibody incubation (Alexa Fluor® 1:500; at room temperature for 1 h). Finally, nuclei were counterstained with DAPI. Primary antibodies used in the present study are reported in the Key Resources Table. Click-iT EdU Cell Proliferation kit for Imaging (Thermo Fisher) was used following the manufacturer’s specifications to identify newly generated cells.

Microscopy
Stained tissue slices were imaged on a Zeiss LSM700 confocal microscope. Tiled images were stitched using a 20% overlap. ImageJ (v1.52r) plugin NeuronJ (v1.4.3) was used to perform a semi-automated reconstruction of the neurites of a few representative Dcx-expressing cells in the cortex (Meijering et al., 2004).

Tissue dissociation and cell sorting
The animals were sacrificed by cervical dislocation at 3-5 wpi and their brains collected in ice-cold Dulbecco’s PBS (D4031, Sigma). Brains were sectioned with a brain slicer into 2 mm-thick coronal slices and, subsequently, micro-dissected to isolate the region subjected to the SWI, or a similar portion of tissue from the healthy contralateral hemisphere (−2 mm²; Figure S1). Brain tissue from several animals was pooled depending on the experimental condition (i.e., Rbpj-kOfl/fl –Injury; Rbpj-kOfl/fl –Healthy; Rbpj-kOfl/fl – Injured; Rbpj-kOfl/fl – Healthy), and enzymatically digested with Miltenyi’s Adult Brain Dissociation Kit, following manufacturer’s specifications.

Finally, tdTomato+ cells were sorted into single cell suspensions on a BD FACS Influx sorter using a 100 μm nozzle, after removal of most CD45+ hematopoietic cells and microglia (CD45-APC/Cy7, 1:200) and Ter119+ erythrocytes (Ter119-APC/Cy7, 1:100).

Library preparation and sequencing
Sampling of cells was performed using Chromium Single Cell 3’ Library & Gel Bead Kit (v2, 10X Genomics). For each sample, cDNA synthesis of sorted single cell suspensions, library preparation, and sequencing were performed at the ESGC facility (Scilife, Stockholm) according to manufacturer’s specifications.

Raw scRNA-seq data processing
Raw reads from Illumina HiSeq2500 or NovaSeq runs were processed using 10X Genomics cellranger standard pipeline (v2.0.1), and mapped to the recommended reference genome (mm10, v2.1.0) supplemented with tdTomato DNA sequence. Raw data from each sample was aligned separately using cellranger count function, and subsequently combined with Seurat R package (v3.3.4) into a unique gene-barcode matrix scaled to a constant factor of 10,000, normalized for sequencing depth, and log-transformed (Butler et al., 2018).

Data filtration and preliminary classification
First, we excluded genes expressed in less than 3 cells. Next, we removed poor quality cells that expressed fewer than 200 unique genes (1), in which mitochondrial content exceeded 20% (2), or lacking expression of Xist (3). We also excluded 49 non-dividing cells.
A total of 5,997 cells were obtained and inspected for further removal of contaminating cells and possible doublets. For this purpose, we ran Seurat’s FindCluster function, a graph-based strategy that identifies cliques using a shared nearest neighbors algorithm, and plotted cells onto the tSNE space (Figure S3E). One astrocyte cluster (AC2) was excluded because of the presence of lower quality cells, counting fewer genes than other clusters (Figure S3G), and only segregating based on the differential expression of widely expressed genes, such as Malat1 and Ckb.

We investigated the expression of classical cell-specific markers throughout the dataset and identified small clusters of microglia (n = 370) and oligodendrocytes (n = 217), which we ignored in downstream analysis for lacking tdTomato expression (< 1 mRNA molecules detected; Figures S3F and S3G). We also recognized a group of VLMCs (n = 374) enriched in tdTomato transcripts due to their expression of Cx30 (Figure S3G), and consequent recombination in our transgenic mouse model. To exclude these cells from downstream analysis of cortical neurogenesis, we performed stab wound injury on animals injected with endoxifen through the cisterna magna, where selective recombination of VLMCs did not trigger cortical neurogenesis, and demonstrating that VLMCs are not a source for the induced neurogenic phenomenon (Figure 2). Following exclusion of contaminating cells, astrocyte and neural progenitor clusters were inspected for the presence of possible doublets based on co-expression of astrocyte- or neural progenitor-specific markers and genes indicative of oligodendrocyte (Cspg4, Mag, and Plip), or endothelial (Car4, Vtn, and Eng) cell identities. A total of n = 4,129 astrocytes and n = 332 neural progenitors were retained for downstream analysis (1986 genes/cell on average; Figure S3H).

**Data Visualization**

Barplots and boxplots used to present quantifications from the immunofluorescence experiments were generated with ggplot2 (v3.0.0) (Wickham, 2016). Seurat and ggplot2 packages were used to generate most graphs for the transcriptional analysis, including violin plots for enriched genes, as well as tSNE/UMAP plots and diffusion maps, which were used to organize cells in lower dimensional spaces. Heatmap in Figures 4D and 5B were generated with monochrome (v2.8.0) (Qiu et al., 2017) and pheatmap (v1.0.12), respectively, whereas the alluvial plot in Figure S6A was generated with alluvial R package (v0.1-2). Z-scores were used for displaying gene expression levels on heatmaps, whereas log-transformed data were used in violin plots and for displaying gene expression levels on tSNE/UMAP embedding.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Quantification of neurogenic cells**

Quantifications included four to seven brains per experimental condition and were performed on four coronal sections spanning the rostro-caudal levels that included the lesion, and focused on the cortical area interested by the injury (1.5 mm²), or a similar surface area on the healthy contralateral hemisphere. Samples from Rbpj-/- null and heterozygous mice were compared on the neurogenic output (i.e., tdTomato⁺ or YFP⁺ cells expressing either Ascl1 or Dcx) using independent measure Student’s t test. Additionally, one-way ANOVA (F statistic) was used to test for differences in neurogenesis across time (i.e., 1, 3, or 8 wpi), and Chi-square test (χ²) was employed to investigate changes in proportions of clusters or single cells at 3 and 8 wpi. Results of the analyses are reported for each comparison indicating the test statistics along with the degrees of freedom specified in brackets, followed by the significance level.

**Dimensionality reduction**

Based on a mean variability plot, displaying average expression and dispersion for each gene, we identified the top 2,512 highly variable genes, which were employed for dimensionality reduction and selection of relevant principal components in downstream analysis. Variance in the dataset was predominantly explained by the first 15 principal components, which were, therefore, used for non-linear dimensionality reduction, as well as for detecting heterogeneous clusters in the dataset.

We plotted the data onto a tSNE space to visualize the phenotypic heterogeneity of astroglial and neural progenitor clusters. In addition, under the assumption of a continuous transcriptional progression that connects astrocytes to their neurogenic progeny, we ran a diffusion map algorithm based on destiny R package (v2.12.0) (Angerer et al., 2016), using as input previously identified variable genes and the top principal components that were not characterized by cell cycle genes (i.e., excluding principal component 2). Furthermore, BuildSNN function was used for constructing a shared nearest neighbor graph plotted onto diffusion map embeddings, which aided identification of the k = 20 nearest neighbors for each cell in the dataset.

**Clustering analysis of astrocytes and progeny**

We included n = 2,512 variable genes to cluster cells using a default graph-based algorithm offered in Seurat package. A first level of unsupervised clustering allowed us to segregate astrocytes and induced neural progenitors. The latter were classified as a unique cluster at a resolution of 0.1, but could be separated into transit amplifying cells (expressing cell cycle-related genes and Ascl1), and postmitotic neuroblasts when sampling at higher resolution (> 0.4, data not shown). Subsequent clustering (res = 0.2) also revealed heterogeneous populations of cortical astrocytes (Figure 3). Astrocyte Cluster 1 (AC1, n = 1,978) and AC2 (1,452) separate based on the genetic manipulation of Rbpj-/-, whereas AC3 expresses reactive glia markers (Figures 3B and 3C).
Pseudotime analysis
We assigned a pseudotime score to each cell based on their projection onto the first diffusion map dimension and used monocle R package (Qiu et al., 2017) to reconstruct the underlying dynamics of gene expression that describe the induced neurogenic program. For this purpose, we ran differential expression analysis and applied a cubic smooth spline model to describe complex patterns of transcriptional fluctuations across pseudotime. Finally, we clustered genes with similar activation dynamics in modules that define the steps undertaken by cortical astrocytes as they commit to a neuronal lineage.

Differential expression and Gene Ontology analyses
We ran pairwise comparisons using Wilcoxon rank sum test between clusters of cells or experimental conditions to identify cell-specific transcriptional profiles. For each comparison we obtained significant DEGs (2108 genes in total for the comparison of AC1-3 and NB, and 129 genes in total for the comparison of AC1 and AC2), which were entered in DAVID bioinformatics database (v6.8). We used Gene Ontology Biological Process and KEGG Pathways as ontology sources to generate lists of enriched terms (> 1.5 enrichment factor), after filtering out entries for which FDR exceeded 15%.

Similarly, we explored Gene Ontology terms resulting from the use of genes that exhibited a relation with pseudotemporal progression, to help defining the transcriptional changes that recapitulate lineage fate transitions. For this purpose, we considered Monocle’s differential expression analysis output and selected the top 1000 genes to use as input for DAVID database search.

Gene regulatory network analysis
For identification and scoring of regulon activity, we employed SCENIC workflow and ran GENIE3 R package (v1.4.3) on log-normalized data to determine sets of co-expressed genes. We, next, linked direct targets to their corresponding transcription factors using RcisTarget databases (v1.2.1), and retained putative downstream genes with enriched DNA motifs at 10kb or 500 bp from the transcription start site (normalized enrichment score > 3). Finally, we used AUCell (v1.4.1) to score activity of each regulon across cells in the dataset, which was computed as the sum of genes expressed per regulon, and produced binary activity matrices based on cut-offs manually adjusted after inspecting the distributions of AUC scores.

Comparison of cortical- and niche-derived neurogenic programs
In order to compare the neurogenic program activated in cortical astrocytes with that described for the adult neurogenic niche, we analyzed scRNA-seq data from the subventricular zone (GEO: GSM3032676, GEO: GSM3032675, and GEO: GSM3032677) (Zywitza et al., 2018) together with the current dataset, and compared the gene expression profiles of cells from the two brain regions. For this purpose, we first we excluded from the subventricular zone dataset cells that are not implicated in the neurogenic program (e.g., oligodendrocytes, immune and vascular cells, ependymal cells, and neurons), based on classical cell type markers, such as Sox10, Csf1r, Vtn, Tac1, and Penk (Figure S5). Subsequently, we ran dimensionality reduction and clustering based on the top 13 principal components as outlined above, and identified populations of astrocytes, neural stem cells, transit amplifying cells, and neuroblasts. Next, we aligned the filtered subventricular zone data to our cortical dataset using canonical correlation analysis (Butler et al., 2018), based on the top 2000 variable genes shared between datasets. Aligned data were visualized with a UMAP plot and re-clustered using FindClusters function on the aligned space. Differential expression analysis was performed as described above to identify enriched markers for each annotate population. Finally, we used AverageExpression function to estimate the average gene expression profile for each cell type in both datasets and computed the correlation to appreciate the similarities between brain regions.
Supplemental Information

A Widespread Neurogenic Potential of Neocortical Astrocytes Is Induced by Injury

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Supplementary Information

Figure S1. Cortical astrocytes upregulate neurogenesis-related markers and enter cell division as they are recruited in the neurogenic program. Related to Figure 1.

(A to D) Representative pictures displaying neurogenic cells found in the injured cortex of \(Rbpj^{-}\) animals. S100-expressing astrocytes upregulate Ascl1 (A) and undergo cell division (B) before generating clusters of neuroblasts (C) and, finally, branching immature neurons (D).

Scale bars represent 20 \(\mu\)m.

(E) Example of a branched Dcx\(^+\) neuroblasts with semi-automated reconstruction of the neurite (NeuronJ). Scale bar represent 25 \(\mu\)m.
Figure S2. Subventricular zone neural progenitors do not contribute to cortical neurogenesis. Related to Figure 2.

(A) Experimental timeline for cortical neurogenesis in the occipital cortex (OC). Rbpj-κ^{fl/fl} mice were treated with tamoxifen on five consecutive days. Four weeks after the last dose of tamoxifen, the animals were subjected to a stab wound injury (SWI) to the occipital cortex (OC). Tissue around the injury site was collected for immunofluorescence (IF) 3 weeks later.

(B) Stitched tile scan illustrating the injured occipital cortex (primary visual cortex, V1). Two clusters of neuroblasts are highlighted in the dashed boxes. Abbreviations: V1, primary visual cortex; CC, corpus callosum; HC, hippocampus. Scale bar represents 100 µm.

(C and D) Magnifications of the boxed areas from B displaying representative examples of YFP+/Dcx+ neuroblasts obtained after inflicting a stab wound injury to the occipital cortex of Rbpj-κ^{fl/fl} mice. Scale bars represent 20 µm.

(E) Stitched tile scan illustrating the labelling achieved with intraventricular delivery of endoxifen. Cx30-expressing cells at, and around, the lateral ventricle (LV) become recombinated with the injection. In the cortex, labelling is restricted to leptomeningeal cells surrounding the surface (arrowhead), while parenchymal astrocytes are spared. The injury site is marked by an asterisk (*). Scale bar represents 500 µm.

(F and G) Labelling of Ascl1^{+}/Ki67^{+} transit-amplifying cells (F) and Dcx^{+} neuroblasts (G) following intraventricular injection of endoxifen. Scale bars represent 20 µm.
Figure S3. Cell census from the single-cell RNA-sequencing experiment on cortical tissue.

Related to Figure 3.
(A) Strategy for microdissecting portions of injured somatosensory cortex and uninjured contralateral tissue from Rbpj-κ null brains and wild-type controls.

(B and C) After tissue dissociation, live cells that were negative for the leukocyte and erythrocyte markers were sorted.

(D) Recombined cells were sorted based on tdTomato expression. Plots represent cells from mice treated with tamoxifen to induce conditional deletion of Rbpj-κ and induction of the fluorescent reporter (left), compared to untreated controls (right), for which we did not detect tdTomato⁺ cells.

(E) Visualization with tSNE discriminates between different cell types composing the dataset, namely astrocytes, neuroblasts, leptomeningeal cells, microglia, and oligodendrocyte. Legend reports total number of cells assigned to each cluster in brackets.

(F) Gene expression levels of classical cell type markers are plotted onto tSNE embedding, thus identifying each populations of cells listed in F.

(G) Violin plots of the total number of detected genes, as well as expression levels for Xist, Gjb6, and tdTomato. Lower number of genes and lack of Xist transcripts motivated exclusion of low-quality cells. Astroglial (Astrocytes 1 and 2) and leptomeningeal cells express Gjb6 (Cx30 gene) transcripts and are, in turn, enriched for tdTomato.

(H) tSNE visualization highlighting cells selected for downstream analysis.
Figure S4. Cells are ordered along the neurogenic and reactive traces using diffusion map.

Related to Figure 4.

(A) Ordering of cells using the top five diffusion map components (dm1 – dm5).

(B) Expression level of genes indicating astroglial (Gja1 and Aqp4), reactive (Bst2) and neurogenic (Sox11, Mcm5, and Cd24a) identities is displayed on a diffusion map embedding.
Figure S5. Cell census of the full subventricular zone dataset. Related to Figure 6.

(A) UMAP visualization of cells encompassing the subventricular zone dataset (Zywitza et al., 2018) split by sequencing run (an002, GSM3032675; an003_F, GSM3032676; an003_L, GSM3032677). Astrocyte and neural progenitor clusters were utilized for downstream analysis and integrated with the current cortical dataset.

(B) Annotation of clusters in A was carried out based on expression of classical cell type.

(C) Violin plots report the total number of genes and the mitochondrial content detected for each cluster. Low quality cells were identified and excluded from subsequent processing based on the lower number of detected features and the higher percentage of mitochondrial genes.
Figure S6. Cortical neurogenic cells cluster together with subventricular zone-borne progenitors and largely share molecular profiles. Related to Figure 6.

(A) Sankey plot describing how previously annotated clusters from the two datasets separately (i.e., cortex and subventricular zone, SVZ, left column) are assigned to the newly computed clusters in the aligned space (right column).

(B) Per-cluster distribution of the experimental conditions from the present study, along with the subventricular zone sample.

(C) Expression of classic astrocyte markers is comparable among astrocyte and neural stem cell clusters.
(D) Violin plots reporting representative neural stem cell markers and showing that subventricular zone cells are enriched in these transcripts, compared to cortical astrocytes co-clustering as neural stem cells.

(E and F) Gene expression levels of classic markers for astrocytes (AC), neural stem cells (NSC), transit-amplifying cells (TAP) and neuroblasts (NB) are displayed onto UMAP embedding in the aligned dataset (G). Activation of the same markers is reported in the dotplot in H to emphasize similar gene activation dynamics between cortical and subventricular zone datasets.

(G) Cortex and subventricular zone differ in expression of a few genes, such as *Id4*, *Dkk3*, *Igfbp2* and *Slc25a18*, which were detected by means of differential expression analysis.

(H) Enrichment of *Id4* and *Igfbp2* transcripts in cortical regions and subventricular zone, respectively, was confirmed with *in situ* hybridization pictures from Allen Brain Atlas’ database.