

**Adult neural stem cells leave the subventricular zone and give rise to
reactive astrocytes in the injured cortex.**

**ADULT NEURAL STEM CELLS FROM THE SUBVENTRICULAR ZONE GIVE
RISE TO REACTIVE ASTROCYTES IN THE CORTEX AFTER STROKE**

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Summary

Reactive astrocytes (RAs) have been reported to convert to multipotent neural stem cells (NSCs) capable of neurosphere (NS) formation and multilineage differentiation *in vitro*. Using genetic tagging, we determined that NSs found in the stroke-injured cortex originate from subventricular NSCs. We demonstrate that these cells can be isolated from the cortex in two different models of stroke, and from different cortical regions. Interestingly, SVZ NSCs give rise to a subpopulation of RAs in the cortex that contributes to astrogliosis and scar formation. Lastly, we show that SVZ-derived RAs can be converted to neurons *in vivo* by forced expression of *Ascl1*. Identifying the contribution of cells originating from the SVZ to injury repair has implications for novel neural regeneration strategies.

Highlights

- NS forming NSCs found in the stroke-injured cortex originate in the SVZ
- SVZ-derived cells differentiate into RAs and contribute to astrocyte scar formation
- A small population of SVZ-derived NSCs persists transiently in the cortex post stroke
- Transcription factor *Ascl1* can convert SVZ-derived RAs to neurons

Introduction

The adult mammalian brain contains a population of NSCs in the SVZ that express Nestin and glial fibrillary acidic protein (GFAP). Adult NSCs give rise to neuroblasts, which migrate to the olfactory bulb (OB) and generate new neurons (Lois and Alvarez-Buylla, 1994). Following brain injury, these neuroblasts can migrate to the damaged site (Arvidsson et al., 2002) following cues released by cells at the injury including reactive glia (Leong and Turnley, 2011). Nevertheless, the brain remains limited in its ability to generate new neurons after injury.

Reactive astrocytes (RAs) are a heterogeneous population of cells (Molofsky et al., 2012) that play key roles in the CNS response to injury, such as restricting inflammation, preventing neuronal loss and repairing the blood brain barrier (Burda and Sofroniew, 2014). Recently, two types of RAs have been defined based on their structural properties: 1) newly proliferated elongated astrocytes that express progenitor markers such as Sox2, BLBP and RC2 and 2) stellate astrocytes that derive from resident cortical astrocytes (Wanner et al., 2013). It has also been suggested that a subset of RAs has the potential to convert to multipotent, self-renewing NSCs capable of NS formation *in vitro* (Buffo et al., 2008; Shimada et al., 2012). However, a recent study has described a novel subpopulation of RAs originating from the SVZ that migrate to the damaged tissue and are distinct from resident RAs in a postnatal mouse model of hypoxia/ischemia (Benner et al., 2013). This led us to question: 1) the

origin of cortical neurospheres after stroke and 2) the contribution of SVZ cells to the astrogliosis that follows brain injury.

We employed genetic tools to address the origin of cortex-derived NSs in two models of stroke injury. Lineage tracking using tamoxifen inducible Nestin-CreER^{T2} (Imayoshi et al., 2006; Lagace et al., 2007) and Cre conditional reporter (Madisen et al., 2010; Srinivas et al., 2001) transgenic mice revealed that cortical NSs originate from SVZ-derived NSCs that migrate to the stroke lesion after injury. These cortical SVZ-derived cells rapidly differentiate and give rise to a subpopulation of RAs that contribute to the astrocyte scar, thus highlighting a role for the SVZ in post injury gliosis. Lastly, we demonstrate that the transcription factor *Ascl1* (also known as *Mash1*) can convert SVZ-derived RAs to neurons, providing a new target for cellular conversion strategies aimed at enhancing neurogenesis.

Results

Neurosphere forming capacity of cells in the injured cortex

To confirm the presence of NSCs in the injured adult cortex, we performed the NS assay on tissue derived from the injured cortex and the SVZ (Figure 1A) of mice four days after endothelin-1 (ET-1) induced stroke. Similar to previous reports (Buffo et al., 2008; Erlandsson et al., 2011; Shimada et al., 2012), NSs formed from the SVZ and stroke-injured cortex (Figure 1B, C), but never from the contralateral (uninjured) cortex (Figure 1C). While the average size of cortex-

derived NSs (ctxNSs) was less than SVZ-derived NSs (svzNSs) (Figure S1A) both populations displayed the cardinal properties of stem cells; self-renewal and multipotency (Potten and Loeffler, 1990). Single neurosphere passaging revealed extended self-renewal (up to passage 5, the longest time examined) with no difference in NS number at each passage (Figure S1B). Furthermore, ctxNSs and svzNSs were multipotent (Figure S1C) with similar differentiation profiles (Figure S1D). We asked if these findings were specific to the site of cortical injury or the type of stroke. In a first series of experiments we assessed NS formation in response to ET-1 strokes in different regions of the cortex. NSs formed from the stroke injured cortex at lesion sites both lateral (Figure 1D,E) and caudal (Figure 1D,E) to the sensory-motor cortex lesions originally examined, revealing that the response was not regionally specific within the cortex. In a second series of experiments we used a different model of stroke, pial vessel disruption (PVD) (Hua and Walz, 2006; Kolb et al., 2007). PVD to the sensory-motor cortex resulted in the generation of cortical derived NSs, identical to what was observed with ET-1 stroke (Figure 1E). Bulk passaging showed that, akin to our original stroke paradigm, NSs from lateral, caudal and PVD stroke sites showed extended passaging (up to Passage 10, the longest time examined) with no difference in NS number between ctxNSs and svzNSs (Figure 1F). Further, no difference was seen in the percent of multipotent ctxNSs versus svzNSs (Figure S1E).

Cortical-derived neurospheres are derived from migratory SVZ stem cells

With the goal of distinguishing between ctxNSs and svzNSs, we took advantage of the Nestin-CreER^{T2};tdTomato^{fl} double transgenic mouse (Imayoshi et al., 2006; Lagace et al., 2007), which enables permanent tdTomato-labeling of cells expressing Nestin during the time of Cre recombinase induction with tamoxifen. Nestin is expressed by neural stem and progenitor cells *in vivo* (Morshead et al., 1994). We crossed these animals with hGFAP-GFP mice that permit visualization of GFAP expressing astrocytes and NSCs (Zhuo et al., 1997). The Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP triple transgenic mice were fed tamoxifen for 2 weeks and chased for 2-3 weeks (Figure 1G), ensuring labeling of SVZ cells with tdTomato and eliminating the possibility of tamoxifen uptake by RAs that express Nestin after stroke (Clarke et al., 1994). Tamoxifen fed triple transgenic mice gave rise to GFP+/tdTomato+ svzNSs while unfed mice gave rise to GFP+/tdTomato- svzNSs (Figure S1F). Most striking, all of the ctxNSs from stroke lesioned, tamoxifen fed Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP mice were GFP+/tdTomato+ (Figure 1G) compared to control unfed mice, where none of the ctxNSs expressed tdTomato. Based on the total number of cells we isolated from the stroke lesioned site, we estimate that approximately 35 ctxNS forming cells are found in the injured cortex at 4d post-stroke. We repeated this experiment in a second strain of Nestin-CreER^{T2} mice (Lagace et al., 2007) to ensure that the findings were not the result of tamoxifen independent tdtomato expression (Imayoshi et al., 2006) or recombination in Nestin-negative cells (Sun

et al., 2014). Nestin-CreER^{T2} mice were crossed with a YFP^{fl} Cre conditional reporter and fed tamoxifen for 2 weeks, followed by a 2-3 week chase (Figure 1H) to label SVZ cells and not RAs with YFP (Figure S1G). Tamoxifen fed mice gave rise to both YFP+ SVZ (Figure 1H) and ctx derived NSs (Figure 1H, Figure S1H). Moreover, YFP+ ctxNSs were seen after ET-1 strokes both lateral and caudal to the original site, as well as after PVD lesions (Figure 1I). Importantly, the frequency of labeling in ctxNSs was similar to the frequency of labeling in the corresponding SVZ (Table S1) supporting the conclusion that ctxNSs are derived from the SVZ.

To confirm the finding that GFAP+ NSCs were giving rise to ctxNSs, we took advantage of the GFAP-TK mice (Bush et al., 1999; Bush et al., 1998) that express herpes simplex virus thymidine kinase (TK) from the GFAP promoter. In these mice, proliferating GFAP+ cells will undergo cell death in the presence of ganciclovir (GCV) (Imura et al., 2003; Morshead et al., 2003). Control (non-TK mice) that received an ET-1 stroke gave rise to ctxNSs in the presence of GCV, however, there was a complete loss of ctxNSs from ET-1 stroke injured mice in the presence of GCV (Figure S1I). These findings are consistent with the conclusion that ctxNSs are derived from GFAP+ NSCs.

To further demonstrate the SVZ origin of ctxNSs, we used a well-established SVZ ablation paradigm (Doetsch et al., 1999) to eliminate proliferating precursors in the SVZ. We predicted that ablating SVZ precursors would result in a loss of ctxNSs at 4 days post ET-1 stroke. Cytosine β -D-

arabinoside (AraC) was intraventricularly infused prior to performing the NS assays. Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP mice received AraC for 10 days prior to stroke and for 4 days post-stroke (AraC (10d+, 4d+), Figure 1J (i)). As previously reported (Doetsch et al., 1999), AraC infusion caused a reduction in the number of svzNSs and as predicted, virtually eliminated ctxNS formation (Figure 1K). We used Ki-67 to assess the possibility that intraventricular AraC administration was depleting the proliferating cells in the injured cortex and this was accounting for the loss of neurosphere forming cells from the cortex. Importantly, we observed Ki67+/GFAP+ cells within the injured cortex demonstrating that the intraventricular AraC infusion did not eliminate resident cortical cells that divide post-stroke (Figure 1L). Hence, the loss of ctxNSs was not due to the AraC kill of mitotically active cells within the lesioned cortex but rather was the result of eliminating proliferating SVZ cells.

Previous studies have shown the rapid repopulation of the SVZ following ablation of the neural precursor pool (Doetsch et al., 1999; Morshead et al., 1994). We reasoned that AraC withdrawal at the time of stroke would permit repopulation of the SVZ and subsequent migration of NSCs to the injured cortex. To test this, AraC was infused for 10 days prior to stroke in triple transgenic mice and the NS assay was performed 4 days later (AraC (10d+, 4d-); Figure 1J(ii)). We observed the return of svzNSs as predicted, but no concomitant increase in the number of ctxNSs on day 4 (Figure 1K). Since the lack of ctxNS formation could be due to a delay in the migration of SVZ cells to the lesion site following

AraC ablation and repopulation, we compared the number of ctxNSs at 7 days post stroke in AraC treated and untreated (stroke only) wild type mice. Interestingly, we found no difference in the numbers of ctxNSs between these groups (Figure S1J). Notably, when we compared the number of ctxNSs at 4 and 7 days in Ara-C untreated stroke-injured mice, the number of ctxNSs at 7 days was significantly less than what was observed at 4 days (Figure 2A). These findings suggest that repopulation of the SVZ occurs at the expense of migrating SVZ cells to the cortex. This may be the result of the cortical microenvironment being less attractive to migrating SVZ precursors at 7 days and/or the microenvironment prompting their differentiation at the site of injury

SVZ stem cells migrate to the stroke site and give rise to reactive astrocytes

Next we examined the time course of migration of SVZ cells to the site of injury. We assayed for ctxNS formation following ET-1 stroke starting at 4 hours post stroke and continuing to 2 months post stroke. As seen in Figure 2A, we observed a gradual increase in the numbers of ctxNS from 1 to 4 days. After 4 days there was a decrease in the numbers of ctxNSs until 10 days and by 2 months virtually no ctxNSs were detected (1 sphere was formed from the cortex in 1 of 8 animals; Figure 2A). Consistent with the migration of SVZ cells to the site of injury, we observed YFP and tdTomato labeled cells (Figure 2B) at 2 and 4 days post stroke in the brain parenchyma of stroke-injured mice. We

characterized the SVZ derived parenchymal cells to determine the proliferation dynamics and phenotype of migratory YFP+ cells. EdU injections were given to NestinCreER^{T2};YFP^{fl} mice for 2 or 4 days after stroke (Figure 2C) and the numbers and locations of YFP+/EdU- and YFP+EdU+ cells were assessed. YFP+ cells were seen in both the corpus callosum and the cortex with no difference in the relative percentage of YFP+Edu+ cells at 2 and 4 days post stroke (Figure 2D). Further we looked for doublecortin (dcx) neuroblasts (YFP+/dcx+) en route to, and/or within, the injury site at 2 and 4 days post stroke and observed a small number of dcx+ cells in the corpus callosum (Figure 2E) at all times examined. However no dcx+ cells were seen in the cortex at 2 days or 4 days post stroke even at a time of peak ctxNS formation (Figure 2E). YFP+ cells were never observed in the parenchyma of control (non-stroked) mice (Figure 2F). To determine if Cspg4+ cells contribute to cortical NS formation, we performed the NS assay in Cspg4-DsRed mice four days after stroke. We never observed DsRed+ ctxNSs (0/6 mice). These findings indicate that neither Cspg4+ cells nor migrating YFP+dcx+ cells give rise to ctxNSs.

Irrespective of ctxNS formation, we consistently observed SVZ derived YFP+ cells in the cortex of stroke injured NestinCreER^{T2};YFP^{fl} mice from 2 days to 2 months post stroke (Figure 2B, 3A). We assessed whether the SVZ-derived cells were contributing to injury-induced astrogliosis. The numbers of GFAP+ astrocytes among SVZ-derived cells from the cortex of Nestin-CreER^{T2};YFP^{fl} mice was assayed at 4, 7 and 10 days post stroke. Indeed, we observed a

significant increase in the percentage of SVZ-derived RAs (YFP+GFAP+) among total SVZ-derived cells (YFP+) from 4 to 7 days and this increase was maintained at 10 days post stroke (Figure 3B). At 2 months, SVZ-derived RAs were seen in the astroglial scar (Figure 3A). To further confirm our findings we used the NestinCreER^{T2};tdTomato^{fl};hGFAP-GFP mice. Quantification of SVZ-derived cells in the cortex at 4, 7 and 10 days after stroke (Figure S2A) showed a decrease in the percentage of GFP- cells among total tdTomato+ cells (Figure S2B) and an increase in the percentage of GFP+ cells among total tdTomato+ cells from 4 days to 7 days (Figure 3B). tdTomato+,GFP+ cells were positive for S100 β , further confirming astrocyte identity (Figure S2C). Given that tamoxifen fed controls also had tdTomato signal in some of the vasculature in the brain (Figure S2D), we may be underestimating the contribution of SVZ derived tdTomato+ cells to reactive astrogliosis. Together, these findings show that SVZ-derived cells migrate to the injury site and differentiate into a population of RAs.

We took advantage of the NS assay to examine the differentiation potential of ctxNS to test the prediction that ctxNS would have a higher propensity to generate astrocyte only clones when isolated at later times post-stroke. Indeed, we found that ctxNSs displayed reduced multipotency when isolated at 7 and 10 days post stroke (88% multipotent neurospheres at 4 days and 13.3% at 10 days post-stroke; Figure 3C). There was a concomitant increase in the number of ctxNSs producing astrocyte only colonies at later times post stroke (7% at 4 days and 80% at 10 days) (Figure 3C), Together, these

findings support the hypothesis that SVZ-derived cells migrate to the injury site post stroke and give rise to RAs.

SVZ-derived reactive astrocytes can be converted into neurons.

We next asked if the SVZ-derived RAs could be converted to neurons. Cultures of RAs were established (Gascon, unpublished) from the stroke lesioned cortex of tamoxifen treated Nestin-CreER^{T2};YFP^{fl} double transgenic mice (Figure 4A). Within the cultures, the GFAP⁺ and S100 β ⁺ astrocytes were invariably YFP⁺, revealing that their origin was exclusively from the SVZ and resident cortical (YFP⁻) astrocytes did not survive in our RA culture conditions (Figure 4B). Given that we observed small numbers of dcx⁺ SVZ derived cells en route to the lesion site post stroke, and the report by Sidenfaden et al. suggesting that neuroblasts can generate glial progeny *in vivo* (Sidenfaden et al., 2006), we sought to determine whether neuroblasts were a source of RAs in our cultures. We did this by MACS sorting PSA-NCAM positive cells (migratory neuroblasts) from the injured cortex and establishing RA cultures from the column flow through (supernatant). RA cultures could not be established from the MACS sorted fraction. To validate the MACS sort, we performed ImageStream analysis on sorted and unsorted fractions from the injured cortex, the RMS (to control for PSA-NCAM⁺ neuroblasts) and the uninjured cortex. ImageStream analysis showed the presence of PSA-NCAM⁺ neuroblasts with intense punctate staining (bright detail intensity) on their surface in the sorted RMS fraction (Figure

4C, Figure S3A) but not in from the damaged cortex (Figure 4C, Figure S3B) or the uninjured contralateral cortex (Figure 4C, Figure S3C), consistent with no RA culture establishment from the sorted cortical fraction and the absence of *dcx+* neuroblasts in the injured cortex at 4d post injury in tissue sections. We confirmed that neurosphere forming cells were not contaminating our RA cultures by plating cells derived from RA cultures in neurosphere forming conditions (Figure 4D). No neurospheres were observed from 1, 2, 3, and 4-day RA cultures (Figure 4E).

To examine whether RAs could be converted to neurons, we established RA cultures from *GFAP-CreER^{T2};YFP^{fl}* mice and forced expression of *Ascl1*, which alone or in combination with other factors has been successfully used to convert fibroblasts, hepatocytes and astrocytes to neurons (Heinrich et al., 2010; Marro et al., 2011; Vierbuchen et al., 2010). We used two different transfection systems: 1) PB-tet-O-*Ascl1*-T2A-BFP and 2) CAG-*Ascl1*-IRES-dsred (Figure 4F). As predicted, we found that forced expression of *Ascl1* resulted in conversion of RAs to *Tuj1+* neurons (6/7 independent cultures showed successful conversion; Figure 4G), with efficiencies ranging from 11-100% (Figure H). We never observed *Tuj1+* neurons in control (non-transfected) cultures (0/6 cultures). *Tuj1+* cells co-expressed the mature neuronal marker *Map2* (Figure S3D), indicating that SVZ-derived RAs can be converted to mature neurons by forced expression of a single transcription factor, *Ascl1*.

To test the possibility of converting SVZ-derived reactive astrocytes to neurons using only *Ascl1* *in vivo*, we transduced GFAP+ reactive astrocytes in tamoxifen fed and chased Nestin-CreER^{T2};YFP^{fl} mice 7 days post stroke with either an AAV5-GFAP-*Ascl1*-T2A-mCherry or an AAV5-GFAP-mCherry control (Figure 4I, Figure S3E). This paradigm allowed us to assess conversion of GFAP+ RAs that were derived from the SVZ (YFP+). Interestingly, at 21 days post stroke, we observed rare YFP+mCherry+dcx+ cells (Figure 4J) and by 35 days YFP+mCherry+NeuN+ cells were seen within the cortex that persisted at 48 days post injury (Figure 4K). Conversion efficiencies using AAV5-GFAP-*Ascl1*-T2A-mCherry were 1.74±1.27% at day 21 and 3.21±1.1% at day 48 (Figure 4L). No NeuN+mcherry+ neurons were detected in AAV5-GFAP-mCherry transduced controls (0/4). Interesting, *in vivo*, *Ascl1* conversion occurs in cells close to the lesion core where the host neurons have undergone cell death. These findings indicate that *Ascl1* alone can be used to reprogram SVZ-derived reactive astrocytes, albeit at low efficiencies, *in vivo*.

Discussion

Using a battery of genetic tools, we have shown that SVZ-derived NSCs migrate to the damaged cortex after injury where a subpopulation will give rise to RAs. While previous studies (Buffo et al., 2008; Shimada et al., 2012) show de-differentiation of reactive astrocytes to multipotent self-renewing cells, we found that cortical neurospheres originate from SVZ-derived precursors. Our triple

transgenic paradigm allowed simultaneous tracking of SVZ precursors that express tdTomato activated by Nestin-CreER^{T2} prior to stroke and GFP driven by the GFAP promoter and astrocytes, which would only express GFP driven by GFAP. No GFP+ only neurospheres were seen, suggesting that RAs do not have the potential to form neurospheres after cortical injury. Rather, we show that NSCs leave their SVZ niche, migrate to the ~~post-injury cortex~~ stroke-injured cortex and retain their ability to form multipotent neurospheres when isolated *in vitro*. These findings were confirmed in NestinCreER^{T2};YFP^{fl} mice, where recombination frequencies in the cortex were similar to what was seen in the SVZ, supporting the hypothesis that ctxNSs originated from neural stem cell migration from the SVZ to the damaged cortex. Further, ablation of dividing cells in the SVZ eliminated ctxNS formation. It remains to be determined whether the differences between the results of these studies are due to the different injury models employed. Stroke elicits a robust response in the SVZ (Arvidsson et al., 2002), while it is not entirely clear if a similar response is seen after stab wound injury (Synowitz et al., 2006; Tzeng and Wu, 1999). There is considerable overlap between markers expressed by NSCs and RAs, such as nestin, GFAP, brain lipid binding protein (BLBP) and vimentin (Robel et al., 2011). Previous studies that performed fate tracking of astrocytes utilized tamoxifen dependent recombination driven by GLAST and GFAP promoters (in GLAST-CreERT2 (Buffo) and GFAP-CreERT2 transgenic mice (Shimada)), both of which are expressed by NSCs in the SVZ (Pastrana et al., 2009; Platel et al., 2009). It remains possible that GLAST

and GFAP recombination occurred in SVZ NSCs and as such, it was SVZ-derived cells that were responsible for the ctxNS formation observed following stab wound or MCAO injury. A number of groups have demonstrated that SVZ precursor cells migrate to areas of injury in the adult brain (Arvidsson et al., 2002; Zhang et al., 2004), and here we have shown that this migration is not limited to progenitor cells using lineage tracking as well as transgenic mice and ablation paradigms that permit the selective loss of neurosphere forming cells. Further, immunohistochemistry and ImageStream analysis revealed that *dcx* expressing progenitors are not present in the cortex at 4d, when we see peak neurosphere formation. Hence, our findings indicate that ctxNSs are derived from SVZ NSCs that migrate to the lesion site post stroke.

Our data suggests that the cortex is permissive for survival of NSC and although there is a rapid decline in NSC in the cortex after injury, a small population transiently survives but is generally absent by 2 months. We show here that one outcome of NSC migration to the damaged cortex is the production of reactive astrocytes. These SVZ-derived reactive astrocytes that contribute to the gliotic response at later times post-injury add to the heterogeneity observed within the reactive astrocyte population and may play specific and important roles in repair following a brain insult. Indeed, a recent study points to specific roles of SVZ-derived RAs in postnatal hypoxia/ischemia; SVZ-derived reactive astrocytes expressed higher levels of thrombospondin-4 and were responsible for proper scar formation and reduction of microvascular hemorrhaging (Benner et al.,

2013). Moreover, a recent article showed no evidence for human cortical neurogenesis after stroke using carbon dating techniques (Huttner et al., 2014) and may point to an alternate role of the SVZ in producing RAs after stroke.

A number of studies have shown that developmental signalling pathways involved in early astroglialogenesis (Bauer et al., 2007; Yang et al., 2013) are reactivated post-injury. (Bauer et al., 2007) Further elucidation of the roles of signalling pathways such as the Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT), and cytokines such as Cardiotrophin-1 (CT-1), Leukemia inhibitory factor (LIF) and Ciliary neurotrophic factor (CNTF) (Yang et al., 2013) will lead to a clearer understanding of the subpopulations of RAs and their involvement in repair mechanisms after stroke. Further, epigenetic modifications that allow binding of transcriptional factors to astrocyte promoters and activate their expression may be the mechanism whereby cytokines exert their effect on astrocyte differentiation (Yang et al., 2013).

The “lack of success” from transplantation paradigms and endogenous neurogenesis (Dibajnia and Morshead, 2013) could be the result of astrocyte differentiation favoured above neuronal differentiation. Indeed, Shimada et.al. showed a loss of neurogenic potential of transplanted cortical derived neurospheres following transplantation back into the uninjured brain; only astrocytes and oligodendrocytes were produced (Shimada et al., 2012). Nonetheless, the existence of a multipotent cell in the cortex after stroke offers the possibility of manipulating these cells for brain repair. Along these lines,

Ascl1 has been reported to promote neurogenesis from NSC and (Kim et al., 2009) astrocytes (Corti et al., 2012) and is a key regulator of fibroblast to neuron conversion *in vitro* (Chanda et al., 2014). We used Ascl1 to target the progeny of SVZ-derived cells and have shown that it is sufficient to convert these cells to neurons, both *in vitro* and *in vivo*. Hence, SVZ-derived reactive astrocytes may provide a novel endogenous target for neural repair. Low conversion efficiency of these cells *in vivo* may reflect regional/age differences in the brain, as has been suggested by recent studies targeting endogenous glia for cellular conversion (Guo et al., 2014; Heinrich et al., 2014; Heinrich et al., 2010; Heinrich et al., 2011; Niu et al., 2013; Pollak et al., 2013; Su et al., 2014). Given the cellular heterogeneity that has been reported, it is likely that cells in different regions may respond differently to transcription factors and other stimuli. Cells that migrate to the cortex, for example, may be intrinsically different or undergo different specification than those that migrate to the striatum, such that transcription factors used successfully in one brain region may not directly translate to another. Indeed, Ascl1 is endogenously upregulated in resident astrocytes in the striatum after stroke (Magnusson et al., 2014) and has been used successfully in combination with other transcription-factors in the striatum (Torper et al., 2013). Ascl1 convert Muller glia into neurogenic progenitors in the retina (Pollak et al., 2013). More importantly, it remains to be seen if manipulating these cells will be beneficial or detrimental to tissue regeneration *in vivo*. A clearer understanding of

subpopulations of RAs and their involvement in repair mechanisms may help us to identify the best cell type for therapeutic cell conversion.

Experimental Procedures

Mice

For NS analysis of cortex-derived versus SVZ-derived NSs at various times post stroke, C57BL/6J or hGFAP^{promoter}-GFP (hGFAP-GFP; Jackson Labs: FVB/N-Tg(GFAPGFP)14Mes/J) (Zhuo et al., 1997) transgenic mice were used. No differences were found between strains regarding neurosphere number, size, passaging and differentiation.

For selective ablation of GFAP+ neurosphere forming cells, GFAP^{promoter}-TK (GFAP-TK; Jackson Labs: B6.Cg-Tg(Gfap-TK)7.1Mvs/J) (Bush et al., 1998) mice were used.

For analysis of the origin of stem cells in the cortex post stroke, Nestin^{promoter}-CreER^{T2} (Nestin-CreER^{T2}) (Imayoshi et al., 2006) transgenic mice were crossed with Rosa26-loxP-stop-loxP-tdTomato (tdTomato^{fl}; Jackson Labs: B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J) (Madisen et al., 2010) transgenic reporter mice and Nestin-CreER^{T2} (Lagace et al., 2007) mice were crossed with Rosa26-loxP-stop-loxP-YFP (YFP^{fl}; Jackson Labs: B6.129X1-Gt(ROSA)26Sortm1(EYFP)Cos/J) (Srinivas et al., 2001) transgenic reporter mice, resulting in Nes-CreER^{T2};tdTomato^{fl} and Nestin-CreER^{T2};YFP^{fl} double transgenic mice. Nestin-CreER^{T2};tdTomato^{fl} mice were crossed to hGFAP-GFP

mice, resulting Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP triple transgenic mice. In addition, Cspg4^{promoter}-DsRed (Cspg4-DsRed; Jackson Labs: Tg(Cspg4-DsRed.T1)1Akik) (Zhu et al., 2008) were used.

To assess conversion of reactive astrocytes to neurons GFAP^{promoter}-CreER^{T2} (GFAP-CrER^{T2}; Jackson Labs: Tg(GFAP-cre/ERT2)13Kdmc) (Casper et al., 2007) were crossed to YFP^{fl} transgenic mice, resulting in GFAP-CreER^{T2};YFP^{fl} transgenic mice.

Both males and females aged 6 weeks – 5 months were included in the analysis. All mice were housed in a barrier facility with a 12 hour light/12 hour dark cycle and allowed free access to food and water with a maximum of 4 mice per cage. Experiments were conducted according to protocols approved by the Toronto Center for Phenogenomics and the Department of Comparative Medicine of the University of Toronto.

Endothelin-1 stroke

Endothelin-1 (ET-1) strokes were performed similar to as previously described (Tennant and Jones, 2009). One μ L of 400 picomolar endothelin-1 (ET-1, Calbiochem) was injected stereotaxically into the cortex at 0.6 AP, 2.25 ML, -1 DV (sensory-motor cortex coordinates), -1.7 AP, +4 ML, -3 DV (lateral coordinates) or -2.3 AP, +2 ML, -0.5 DV (caudal coordinates), relative to bregma. A 26 gauge Hamilton syringe with a 45 degree beveled tip was used to inject ET-

1 at a rate of 0.1ml/min. The needle was left in place for 10 minutes after ET-1 injection to prevent backflow and then slowly withdrawn.

PVD stroke

PVD strokes were induced by removing the skull and dura in the region bound by 0.5 to +2.5 AP, +0.5 to 3 ML, relative to bregma. A saline-soaked cotton swab was used to remove pial vessels.

Cytosine b-D-arabinoside (AraC) infusion

For AraC infusion, a cannula was implanted to target the lateral ventricle (0.2 AP, 0.8 ML, and 2.5 DV, relative to bregma) and connected to a subcutaneous mini-osmotic pump (1007D, Alzet; 0.5 μ l/hour) overlying the shoulder blade and lateral to the spine. 2% AraC was delivered for 10-14 days to ablate cycling cells in the SVZ.

Tamoxifen induction

For tamoxifen induction *in vivo*, at or after 6 weeks of age, animals were fed tamoxifen food (Harlan) for 2 weeks and then switched to a high fat diet during a chase period of 2-3 weeks. Tamoxifen food was custom formulated to contain 0.5% TAM and 5% sucrose by weight and blue dye (to enable monitoring of consumption based on color of feces) in a high fat base (Harlan Diet 2019).

For tamoxifen induction *in vitro*, 1mM OH-Tamoxifen (H7904, Sigma) was added every other day, for 7 days.

Adeno Associated Virus (AAV) Viral injections

AAV5-GFAP(0.7)^{promoter}-mCherry-T2A-mAscl1-WPRE (AAV5-GFAP-mCherry-T2A-Ascl1; 3.9×10^{13} GC/mL) and AAV5-GFAP(0.7)^{promoter}-mCherry-WPRE (AAV5-GFAP-mCherry; 2.9×10^{13} GC/mL) particles were purchased from VectorBioLabs. 1uL of AAV was injected into the injured cortex of NestinCreER^{T2};YFP^{fl} mice 7d post stroke at a rate of 0.1mL/min at the following coordinates: 0.6 AP, 2.25 ML, -1 DV, 1.6 AP, 2.25ML -1 DV and -1 AP, 2.25 ML, -1 DV, relative to bregma. The needle was left in place for 10 minutes after AAV injection to prevent backflow and then slowly withdrawn.

5-ethynyl-2'-deoxyuridine (EdU) injections

250 mg/kg EdU (Life Technologies, E10187) was injected intraperitoneally once daily for either 2 or 4 days after stroke.

Tissue processing, immunohistochemistry and quantification.

Mice were anesthetized with Avertin, perfused transcardially and postfixed for 2 hours with 4% paraformaldehyde. Brains were cryopreserved in 30% sucrose, frozen and 18 μ m coronal cryostat sections were cut.

Cryosections were blocked with 10% normal goat serum (NGS) and 0.3% triton in PBS and labeled with a primary antibody (Table 2) in PBS overnight at 4°C, followed by incubation with a secondary antibody and a nuclear stain (Table 2) in PBS for 1h at room temperature (RT). For double and triple immunohistochemistry, sections were reblocked in 10% NGS and 0.3% triton in PBS, incubated with a primary antibody 4°C O/N and then with a secondary antibody at RT for 1h. Samples labeled with gt anti dcx primary antibody were blocked in 10% normal donkey serum and 0.3% triton in PBS and incubated O/N at RT. Samples labeled with anti Ch Alexa 488 secondary antibody were incubated for 2h at RT. For EdU staining, a ClickiiT EdU imaging kit (Life Tech, C10337 and C103040) was used according to the manufacturer's instructions.

In vivo, overlapping expression of tdTomato and GFP or YFP and GFAP in cell bodies was quantified in three 40x confocal stacks in 3 coronal sections per animal. For quantification of EdU or dcx and YFP, three 20x confocal stacks in 3 coronal sections per animal were obtained. For analysis of mCherry, YFP and neuronal markers, at least 100 cells were counted in at least three 20x confocal stacks per animal. Imaging was performed using Zen 2011 software and a Zeiss two-photon microscope, Velocity software and a Zeiss spinning disc confocal or Axiovision software and a Zeiss Observer Z1 inverted fluorescence microscope. Linear adjustments of contrast and brightness were made to micrographs using the respective microscope software. Threshold intensity was set according to the background signal detected in controls.

Table 2. Summary of reagents used for immunohistochemistry

Primary Antibodies	Dilution	Company	Catalogue Number
Ms anti- β -III-tubulin (Tuj1)	1:500	Covance	MMS-435P-250
Rb anti- β -III-tubulin (Tuj1)	1:500	Covance	MRB-435P-100
Rb anti-GFAP	1:3000	DAKO	2016-04
Rb anti-S100 β	1:200	DAKO	Z0311
Ms anti MAP2(a+b)	1:200	Sigma	M1406
Ms (IgM) anti O4	1:1000	R&D Systems	MAB1326
Rb anti Ki67	1:200	AbCam	Ab15580
Ch anti GFP	1:1000	Aves	GFP-1020
Rb anti RFP	1:2000	Rockland	600-401-379
Rb anti NeuN	1:100	Millipore	ABN78
Gt anti dcx	1:200	Santa Cruz	sc-8066
Secondary Antibodies	Dilution	Company	Catalogue Number
Anti ms IgM Alexa 568	1:400	Life Tech	A21043

Anti-ms IgG Alexa 568	1:400	Life Tech	A11031
Anti-ms IgG Alexa 488	1:400	Life Tech	A11001
Anti ms IgG Alexa 647	1:400	Life Tech	A21236
Anti gt IgG Alexa 647	1:400	Life Tech	A21447
Anti rb Alexa 568	1:400	Life Tech	A11036
Anti rb Alexa 647	1:400	Life Tech	A21245
Anti ch Alexa 488	1:400	Life Tech	A11039
Nuclear Stains	Dilution	Company	Catalogue Number
DAPI	50ng/mL	Life Tech	D3571
Topro	1:1000	Life Tech	T3605

Neurosphere Culture

Adult mice were anesthetized with isoflurane and cervically dislocated. Animals were decapitated and the meninges were removed. For cortical dissections, coronal slices were made using a scalpel blade and the cortex was collected, taking care to avoid the corpus callosum. The SVZ was dissected as previously described (Coles-Takabe et al., 2008; Tropepe et al., 1997). The tissue was enzymatically and mechanically dissociated into a single-cell suspension. Cells were plated at clonal density (5 cells/ μ L) in NS media

(Neurobasal (Life Tech) containing L-glutamine (2 mM, Life Tech), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Life Tech), B27 (1:50, Life Tech), epidermal growth factor (EGF; Sigma; 20ng/ml), fibroblast growth factor (FGF; Sigma; 10ng/ml), and heparin (2000ng/ml). For gangciclovir (GCV) ablation experiments, 20 μ M GCV was added to the media before plating. After 10 days *in vitro*, the numbers of primary NSs was counted and the size of individual NSs was measured using Image J software (NIH). Primary NSs were grown for 10 days. For single sphere passaging, individual NSs were passaged every 7 days. For bulk sphere passaging, NSs were passaged every 7 days. NSs were mechanically dissociated into a single-cell suspension, and replated under the same conditions as primary cultures. The number of ctxNSs per mouse was calculated as follows:

Number of cortical neurosphere forming cells per brain at 4d post-stroke =
(number of cells after dissociation x number of neurospheres per well) / number
of cells per well

Neurosphere differentiation immunocytochemistry and quantification.

One primary NS was plated per well of a 48-well plate coated with laminin (5 μ g/ml, Sigma) in differentiation medium (Neurobasal, Life Tech) containing L-glutamine (2 mM, Life Tech), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Life Tech), B27 (1:50, Life Tech) and 1% fetal bovine serum (Life Tech). After 7 days of differentiation, cells were fixed with 4% paraformaldehyde.

For immunocytochemistry for cell phenotype markers, cell cultures were fixed in 4% PFA, blocked with 10% NGS in PBS and labeled with mouse anti-O4 (Table 2) overnight (O/N) at 4°C followed by an incubation with a secondary antibody (Table 2). For double and triple immunolabeling, cultures were re-blocked in 10% NGS and 0.3% triton in PBS and incubated with rabbit anti-GFAP and mouse anti- β -III-tubulin (Table 2) followed by incubation with a secondary antibody and DAPI (Table 2). The number of phenotype marker positive cells and DAPI positive cells were quantified in three 10x photos per well. Images were captured using Zen 2011 software and a Zeiss two-photon microscope or a Zeiss inverted microscope.

Reactive astrocyte culture

GFAP-CreER^{T2};YFP^{fl} adult mice were anesthetized with isoflurane and cervically dislocated at 4d post-stroke. The meninges were removed and coronal slices were made using a scalpel blade. The cortex was collected, taking care to avoid the corpus callosum. The tissue was enzymatically and mechanically dissociated into a single-cell suspension. One cortical hemisphere was resuspended in 1mL of astrocyte media (DMEM/F12 (Life Tech) containing L-glutamine (2 mM, Life Tech), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Life Tech), B27 (1:50, Life Tech), 0.45% glucose (Sigma, G8769) and 10% fetal bovine serum (FBS, Life Tech)) and plated in 2 wells of a 24 well poly-d-lysine

coated plate. Media was changed the next day. Cultures could only be established from the ipsilateral hemisphere and never from the hemisphere contralateral to the stroke site.

Astrocyte conversion in vitro

Two days after reactive astrocyte culture establishment from GFAP-CreER^{T2};YFP^{fl} mice, 4-Hydroxytamoxifen (4-OH-TAM) was added to astrocyte media. Media + 4-OH-TAM was changed every other day for 5d. After 7d, cells were transfected with PBase, PB-CAG-rtTA (Woltjen et al., 2009) and PB-tetO-Ascl1-T2A-BFP or CAG-Ascl1-IRES-dsred using HD Fugene (Roche) at a ratio of 2:1. For control experiments either PB-tet-O-BFP or CAG-dsred was used. The media was changed to N2B27 (50% Neurobasal (Life Tech) and 50% DMEM/F12 (Life Tech) containing B27 (1:50, Life Tech) and N2 (1:100, Life Tech). For PB-tetO-Ascl1-T2A-BFP expression, 1500 ng/mL dox was added to the media. Media was changed every other day for the duration of the experiment.

The number of neurons was quantified by assessing converted neurons (Tuj1+YFP+BFP+ or Tuj1+YFP+dsred+ cells)/transfected astrocytes (YFP+BFP+ or YFP+dsred+ cells).

Plasmids

PB-tetO-Ascl1-T2A-BFP

Fusion PCR was used to amplify Ascl1 and BFP from Tet-O-FUW-Ascl1 (Addgene) and pENTR-BFP and generate an attb PCR product with a T2A linker between Ascl1 and BFP and gateway overhangs using the following primers:

Attb_Ascl1_Fwd

**5'GGGGACAAGTTTGTACAAAAAAGCAGGGTTCGCCACCATGGAGCAAAGC
TCATTT3'**

Ascl1_T2A_Rev

5'CCTCTGCCCTCTCCGGATCCGAACCAGTTGGTAAAGTCC3'

T2A_BFP_Fwd

**5'GGATCCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGG
AGAATCCTGGCCCCATGGTGAGCAAGGGCGAG3'**

BFP_Attb_Rev

5'GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACTTGTACAGCTGCTC3'

The attb PCR product was cloned using Gateway into pRetroX (Life Tech) and Piggybac transposon destination vectors. Vectors were sequenced for verification.

CAG-Ascl1-IRES-dsred and *CAG-dsred* were kind gifts from Magdalena Gotz.

MACS and Image Stream FACS Analysis

Adult mice were anesthetized with isoflurane and cervically dislocated. Animals were decapitated at 4d post-stroke. The meninges were removed and

coronal slices were made using a scalpel blade. The cortex was collected, taking care to avoid the corpus callosum. The RMS was also dissected. The tissue was enzymatically and mechanically dissociated into a single-cell suspension. Cells were resuspended in MACS sorting buffer (Ca^{++} and Mg^{++} free PBS with 2% FBS and 1mM EDTA), stained with anti-PSA-NCAM-PE (1:100, Miltenyi Biotech, 30-093-274) and MACS sorted using a PE selection kit (Stem Cell Technologies, 18554) and an EasySep Magnet (Stem Cell Technologies, 18000), according to the manufacturer's instructions. For ImageStream analysis, dissociated cells were resuspended in 500uL of PBS (azide and serum/protein free) and stained with a fixable cell viability dye (FVD eFluor 780; eBioscience, 65-0865-14) according to the manufacturer's instructions. Cells were then washed with 10% FBS in PBS, resuspended in 500 μL of MACS sorting buffer, stained with anti-PSA-NCAM-PE (1:100, Miltenyi Biotech, 30-093-274) or a mouse IgM-PE control (1:100, Miltenyi Biotech, 130-099-127), MACS sorted using a PE selection kit (Stem Cell Technologies, 18554) and an EasySep Magnet (Stem Cell Technologies, 18000), according to the manufacturer's instructions, stained with Hoescht (1 $\mu\text{g}/\text{mL}$, BD Biosciences, 33342) for 30 min at RT and resuspended in MACS sorting buffer. Cells from the MACS sorted fractions, column flow through (supernatant) fractions and unsorted cells from the injured cortex, uninjured (contralateral) cortex and RMS were analysed with an Amnis ImageStreamTM Mark II imaging flow cytometer (AMNIS). Single stained controls were collected for the compensation matrix. The resulting compensated image files were

analysed using IDEAS analysis software (AMNIS). Focused, single cells were selected based on viability (exclusion of FVD e780 dye) and the presence of a nucleus (positive for Hoescht staining). The live nucleated cells were examined for PE-PSA-NCAM signal and the specific and punctate PSA-NCAM-PE binding to cells was described using the Bright Detail Intensity feature of the IDEAS software. The increased Bright Detail Intensity scores differentiated PSA-NCAM-PE binding from non-specific, diffuse binding of the IgM-PE isotype control. The antibody binding patterns were confirmed in composite cell images of bright field and PE fluorescence generated from the ImageStream data.

Analysis of the RMS supernatant showed high purity (only one cell escaped the column).

Data Acquisition and Statistical Analysis

All experiments were conducted blind, using a numbering system. F tests were used to compare variance between groups. Unpaired two-tailed *t* tests or unpaired two-tailed *t* tests with Welch's correction (for unequal variance) were used for comparisons between two groups. One-factor ANOVA was used followed by post hoc analysis (Tukey's or Dunnet's test) for comparisons between three or more groups. Comparison of percentages between groups was done using two sample z-tests for individual proportions. Differences were considered significant at $p < 0.05$. Values are presented as mean \pm SEM.

Author contributions

M.F. and N.S. conceived, designed and conducted experiments and analyzed and interpreted data. S.G. designed and characterized the reactive astrocyte culture system. K.W.A.B. designed and conducted ImageStream experiments and analyzed and interpreted data. C.M. and A.N. conceived and designed experiments, provided financial support, analyzed and interpreted data. M.F. assembled data and wrote the manuscript. All authors contributed to the final version of the manuscript.

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Figure Legends

Figure 1. Migratory SVZ cells give rise to neurospheres in the cortex.

(A) Experimental design for cortical NS culture. (B) SVZ-derived and cortical-derived neurospheres (svz and ctx) (C) Number of NSs formed from the uninjured and injured cortex and SVZ (** = $p=0.001$, unpaired *t* test with Welch's correction, $t(8)=5.006$, data represent \pm s.e.m., $n_{ctx}=11$ mice, $n_{svz} = 9$ mice). (D) Diagram of injection sites lateral and caudal injection to the original sensory-motor cortex lesion site (E) Number of neurospheres formed from the cortex and SVZ in lesions lateral and caudal to the original sensory-motor cortex lesion (smctx) and after pial vessel disruption (PVD) (***= $p<0.001$, unpaired *t* test, $t(12)=9.785$, data represent \pm s.e.m., $n_{smctx_ctx}= 9$ mice, $n_{smctx_SVZ} = 5$ mice; ***= $p<0.001$, unpaired *t* test, $t(6)=8.454$, data represent \pm s.e.m., $n_{lat_ctx}= 4$ mice, $n_{lat_SVZ} = 4$ mice; *= $p<0.05$, unpaired *t* test, $t(4)=3.908$, data represent \pm s.e.m., $n_{caud_ctx}= 3$ mice, $n_{caud_SVZ} = 3$ mice; *= $p<0.05$, unpaired *t* test, $t(12)=2.978$, data represent \pm s.e.m., $n_{PVD_ctx}=7$ mice, $n_{PVD_SVZ} = 7$ mice). (F) Number of

neurospheres at passage 4 and passage 10 from the original sensory-motor cortex lesion, lateral and caudal to that site and after PVD (n.s., unpaired *t* test, $t(5)_{\text{passage4_et-1}} = 0.1879$, $t(11)_{\text{passage10_et-1}} = 1.1016$, $t(12)_{\text{passage4_PVD}} = 0.3755$, $t(10)_{\text{passage10_PVD}} = 1.910$, $t(3)_{\text{passage4_lat}} = 0.07205$, $t(2)_{\text{passage10_late}} = 0.112$, $t(4)_{\text{passage4_caud}} = 1.349$, $t(4)_{\text{passage10_caud}} = 0.9843$, data represent \pm s.e.m., $n_{\text{P4_et1_ctx}} = 3$, $n_{\text{P10_et1_ctx}} = 6$, $n_{\text{P4_PVD_ctx}} = 7$, $n_{\text{P10_PVD_ctx}} = 7$, $n_{\text{P4_lat_ctx}} = 2$, $n_{\text{P10_lat_ctx}} = 2$, $n_{\text{P4_caud_ctx}} = 2$, $n_{\text{P10_caud_ctx}} = 2$, $n_{\text{P4_et1_SVZ}} = 4$, $n_{\text{P10_et1_SVZ}} = 7$, $n_{\text{P4_PVD_SVZ}} = 6$, $n_{\text{P10_PVD_SVZ}} = 6$, $n_{\text{P4_lat_SVZ}} = 3$, $n_{\text{P10_lat_SVZ}} = 2$, $n_{\text{P4_caud_SVZ}} = 4$, $n_{\text{P10_caud_SVZ}} = 4$, 3 independent trials). (G) Lineage tracing of SVZ cells using Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP mice. Picture shows tdTomato expression in ctxNSs. (H) Lineage tracing of SVZ cells using Nestin-CreER^{T2};YFP^{fl} mice. Pictures show YFP expression in ctxNSs and SVZNSs. (I) YFP expression in ctxNSs from stroke sites lateral and caudal to the original sensory-motor cortex lesion site and PVD. (J) AraC ablation paradigms. (K) Number of ctxNSs and svzNSs after AraC ablation (** = $p < 0.01$, *** = $p < 0.001$, One-way ANOVA with Tukey's post hoc, $F(2,11) = 16.91$, data represent \pm s.e.m. from $n_{\text{saline}} = 8$, $n_{14\text{dAraC}} = 4$, $n_{10\text{dAraC}} = 2$). (L) Ki-67 positive cells in the cortex. uninj = uninjured, inj = injured, SVZ = subventricular zone, lv = lateral ventricle, ctx = cortex, cc = corpus callosum, smctx = sensory-motor cortex, PVD = pial vessel disruption, lat = lateral, caud = caudal, tdTom = tdTomato, TAM = tamoxifen

Figure 2. Characterisation of migratory SVZ cells.

(A) Number of ctxNSs (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, One-way ANOVA with Tukey's post hoc, $F(9,70)_{ctx} = 7.669$, $F(9,61)_{svz} = 1.032$, data represent \pm s.e.m , $n_{0dcx} = 3$, $n_{4h_cx} = 4$, $n_{1d_cx} = 7$, $n_{2d_cx} = 6$, $n_{3dcx} = 7$, $n_{4d_cx} = 21$, $n_{7d_cx} = 7$, $n_{10d_cx} = 8$, $n_{2mo_cx} = 8$). (B) SVZ-derived cells migrating to the cortex in Nestin-CreER^{T2};YFP^{fl} and Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP mice. (C) EdU labeling paradigm in Nestin-CreER^{T2};YFP^{fl} mice. (D) YFP+EdU+ and YFP+EdU- cells in the cortex and corpus at 2d and 4d after stroke. Inset shows higher magnification of boxed cell. (n.s. = $p_{ctx_2d_vs_4d} = 0.6644$, unpaired t test, $t(8) = 0.4504$, data represent \pm s.e.m., $n_{2d_ctx} = 5$ mice, $n_{4d_ctx} = 6$ mice; n.s. = $p_{cc_2d_vs_4d} = 0.0883$, unpaired t test with Welch's correction, $t(5) = 2.113$, data represent \pm s.e.m., $n_{2d_cc} = 4$ mice, $n_{4d_cc} = 6$ mice. (E) YFP+dcx+ and YFP+dcx- cells in the cortex and corpus callosum at 2d and 4d after stroke. (F) YFP expression in tamoxifen fed, uninjured controls. White and yellow insets show higher magnification photos of regions in dashed white and yellow boxes. ctx = cortex, cc = corpus callosum, SVZ = subventricular zone, TAM = tamoxifen.

Figure 3. Migratory SVZ cells give rise to reactive astrocytes at the lesion site.

(A) Lineage tracing of SVZ cells using Nestin-CreER^{T2};YFP^{fl} mice. YFP+ cells in the parenchyma from 4 days to 2 months after stroke. (B) Ratio of SVZ-derived reactive astrocytes ($YFP+GFAP+/YFP^{total}$ or $Tom+GFP+/Tom^{total}$) in Nestin-CreER^{T2};YFP^{fl} and Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP mice (* = $p < 0.05$ *** = $p < 0.001$, One-way ANOVA with Tukey's post hoc, $F(3,19)_{YFP+GFAP+/YFP^{total}} =$

19.87, data represent \pm s.e.m., $n_{4d} = 6$ mice, $n_{7d} = 7$ mice, $n_{10d} = 7$ mice); One-way ANOVA with Tukey's post hoc, $F(3,10)_{Tom+GFP+/Tom^{total}} = 5.5852$ data represent \pm s.e.m., $n_{4d} = 3$ mice, $n_{7d} = 4$ mice, $n_{10d} = 4$ mice). (C) Percentage of multipotent, bipotent and astrocyte only cortex-derived NSs (** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ Z-test for independent proportions, $Z_{4dvs7dmultipotent} = 3.123$ $Z_{4dvs10dmultipotent} = 3.9155$, $Z_{7dvs10dmultipotent} = 0.8007$, $Z_{4dvs7dastrocyte_only} = 3.5543$ $Z_{4dvs10dastrocyte_only} = 3.9506$, $Z_{7dvs10dastrocyte_only} = -0.3105$, data represent $n_{4d} = 14$ neurospheres, $n_{7d} = 12$ neurospheres, $n_{10d} = 15$ neurospheres. Tom = tdTomato, SVZ = subventricular zone, lv = lateral ventricle, ctx = cortex, cc = corpus callosum, TAM = tamoxifen.

Figure 4. Forced transcription factor expression converts SVZ-derived reactive astrocytes to neurons.

(A) Lineage tracing of SVZ-derived reactive astrocytes *in vitro* using Nestin-CreER^{T2};YFP^{fl} mice. (B) YFP expression in GFAP+ and S100 β + astrocytes. (C) Pictures from ImageStream analysis of MACS sorted RMS, injured cortex and uninjured contralateral cortex. (D) Experimental design for assessing the presence of a residual precursor cell in RA culture conditions. (E) Numbers of neurospheres derived from plating cortex and SVZ in RA conditions. (F) Experimental design for conversion using GFAP-CreER^{T2};YFP^{fl} mice. Picture shows YFP+ astrocytes after 4-OH-TAM induced recombination (G) Tuj1+ neuron after expression of PB-tet-O-Ascl1-T2A-BFP in YFP labeled astrocytes *in*

vitro. (H) Percent neuronal conversion. Blue dots represent experiments using PB-tet-O-Ascl1-T2A-BFP (n=3; Tuj1+YFP+BFP+ cells/YFP+BFP+ cells). Red dots represent experiments using CAG-Ascl1-IRES-dsred (n=3; Tuj1+YFP+dsred+ cells/YFP+dsred+ cells) (I) Experimental design for *in vivo* conversion using Nestin-CreER^{T2};YFP^{fl} mice. (J) Dcx+ neuroblasts after expression of AAV5-GFAP-Ascl1-T2A-mCherry. Higher magnification is shown of the boxed cell. (K) NeuN+ neurons after expression of AAV5-GFAP-Ascl1-T2A-mCherry. Higher magnification is shown of the boxed cell. (L) Percent neuronal conversion (n=4, mCherry+dcx+/mCherry+; n=4 mCherry+NeuN+/mCherry+). TAM = tamoxifen, dox = doxycycline, 4-OH-TAM = hydroxytamoxifen. ctx = cortex, RMS = rostral migratory stream, BF = brightfield

Figure S1, Related to Figure 1. Neurospheres derived from the cortex

(A) Size of ctxNSs (** =p=0.0011, unpaired *t* test with Welch's correction, $t(135) = 3.343$, data represent \pm s.e.m., $n_{ctx} = 71$, $n_{SVZ} = 72$, 4 independent trials). (B) Number of neurospheres from individual clones at each passage (n.s., unpaired *t* test with Welch's correction (except passage 2), $t(75)_{\text{passage1}} = 1.528$, $t(71)_{\text{passage2}} = 0.4922$, $t(55)_{\text{passage3}} = 0.3393$, $t(31)_{\text{passage4}} = 1.254$, $t(19)_{\text{passage5}} = 1.161$ data represent \pm s.e.m., $n_{P1ctx} = 73$, $n_{P2ctx} = 39$, $n_{P3ctx} = 49$, $n_{P4ctx} = 27$, $n_{P5ctx} = 9$, $n_{P1SVZ} = 17$, $n_{P2SVZ} = 16$, $n_{P3SVZ} = 16$, $n_{P4SVZ} = 16$, $n_{P5SVZ} = 16$, 4 independent trials). (C) Multipotent sphere with Tuj1+ neurons, O4+ oligodendrocytes and GFAP+ astrocytes. (D) Percentage of neurons, astrocytes and oligodendrocytes upon

differentiation (n.s. unpaired *t* test, $t(18)_{Tuj1} = 1.325$, $t(17)_{O4} = 1.4225$, $t(8)_{GFAP} = 0.02691$, data represent \pm s.e.m. from $n_{Tuj1_cx} = 16$, $n_{Tuj1_SVZ} = 4$; $n_{Tuj1_cx} = 13$, $n_{Tuj1_SVZ} = 6$; $n_{O4_cx} = 7$, $n_{O4_SVZ} = 3$, 3 independent trials). (E) Percent multipotent neurospheres formed from the cortex after lesions at sites lateral and caudal to the original injury of the sensory-motor cortex and from the cortex after pial vessel disruption (PVD) ($n_s = p_{PVD} = 0.18684$, $p_{lateral} = 0.90448 = p_{caudal} = 0.31732$ Z-test for independent proportions, $z_{PVD} = -1.3242$ $z_{lateral} = 0.124$, $z_{caudal} = 1.0022$, data represent $n_{lateral_ctx} = 11$, $n_{lateral_SVZ} = 12$, $n_{caudal_ctx} = 4$, , $n_{caudal_SVZ} = 14$, $n_{PVD_ctx} = 16$, $n_{PVD_SVZ} = 28$). (F) tdTomato expression in svzNSs from tamoxifen fed and unfed mice. (G) Nestin expression in YFP-GFAP+ astrocytes 4d after stroke. (H) YFP expression in ctxNSs from tamoxifen fed mice. (I) GCV ablation paradigm and number of cortex-derived neurospheres after GCV administration in wt and GFAP-TK mice (**= $p = 0.0015$, paired *t* test, $t(4) = 7.770$, data represent \pm s.e.m. from $n_{wt_+GCV} = 5$, $n_{wt_ -GCV} = 5$; n.s. = $p = 0.3503$, paired *t* test, $t(5) = 1.030$, data represent \pm s.e.m. from $n_{GFAP-TK_+GCV} = 6$, $n_{GFAP-TK_ -GCV} = 6$). (J) Number of ctxNSs at 7d and 7d after AraC ablation (n.s., unpaired *t* test, $t(9) = 1.857$, data represent \pm s.e.m. from $n_{10dAraC_7d} = 4$, $n_{7d} = 7$. SVZ = subventricular zone, cc = corpus callosum, ctx = cortex, lv = lateral ventricle, PVD = pial vessel disruption, TAM = tamoxifen, tdTom = tdTomato.

Figure S2, Related to Figure 3. SVZ-derived cells differentiate into reactive astrocytes in the cortex after stroke.

(A) tdTomato+ and GFP+ cells at 4d and 7d post stroke. White arrow shows a tdTomato+GFP- cell, white asterisks show tdTomato+GFP+ cells. (B) Ratio of SVZ-derived precursors ($\text{Tom+GFP+}/\text{Tom}^{\text{total}}$) in Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP mice (* = p<0.05, One-way ANOVA with Tukey's post hoc, $F(3,10)_{\text{Tom+GFP-}/\text{Tom}^{\text{total}}_+} = 8.442$, $F(3,10)$ data represent \pm s.e.m., $n_{4d} = 3$, $n_{7d} = 4$, $n_{10d} = 4$). (C) S100 β expression in tdTomato+GFP+ reactive astrocyte. White arrowheads show tdTomato+GFP+S100 β + positive cell, yellow arrowheads show tdTomato-GFP+S100 β + cells. (D) tdTomato expression in uninjured tamoxifen fed mice. White and yellow insets show higher magnification photos of regions in dashed white and yellow boxes, asterisks show labeling of vasculature. ctx = cortex, cc = corpus callosum, dcx = doublecortin, SVZ = subventricular zone, Tom + tdTomato.

Figure S3, Related to Figure 4. Conversion of reactive astrocytes to neurons

(A) ImageStream plot of the MACS sorted RMS. Gated cells in R5 show punctate PSA-NCAM on the surface of cells in the RMS. (B) ImageStream plot of the MACS sorted injured cortex. (C) ImageStream plot of the MACS sorted uninjured contralateral cortex. (D) Tuj+Map2+ neuron converted from a YFP labeled astrocyte. (E) mCherry expression in the cortex after AAV5-GFAP-Ascl1-T2A-mCherry injection. ctx = cortex