Emx1-expressing neural stem cells in the subventricular zone give rise to new interneurons in the ischemic injured striatum

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Abstract

Neural stem cells from different regions within the subventricular zone (SVZ) are able to produce several different subtypes of interneurons in the olfactory bulb throughout life. Previous studies have shown that ischemic stroke induces the production of new neurons in the damaged striatum from the SVZ. However, the origins and genetic profiles of these newborn neurons remain largely unknown as SVZ neural stem cells are heterogeneous. In the present study, using a mouse model of perinatal hypoxic-ischemic (H/I) brain injury combined with BrdU labeling methods, we found that, as in rat brains, virtually all newborn neuroblasts that migrate from the SVZ into the ischemic injured striatum exclusively express the transcription factor Sp8. Furthermore, although newborn neuroblasts are plentiful in the damaged striatum, only a few can differentiate into calretinin-expressing (CR+) interneurons that continuously express Sp8. Genetic fate mapping reveals that newly born CR+ interneurons are generated from Emx1-expressing neural stem cells in the dorsal–lateral SVZ. These results suggest that the fate of the Emx1-expressing lineage in the ischemic damaged striatum is restricted. However, when Sp8 was conditionally inactivated in the Emx1-lineage cells, Pax6 was ectopically expressed by a subpopulation of Emx1-derived CR+ cells in the normal and damaged striatum. Interestingly, these cells possessed large cell bodies and long processes. This work identifies the origin of the newly born CR+ interneurons in the damaged striatum after ischemic brain injury.

Introduction

The existence of neural stem cells and the persistence of neurogenesis in the postnatal subventricular zone (SVZ) (Ming & Song, 2005; Zhao et al., 2008; Kriegstein & Alvarez-Buylla, 2009) have raised hopes for the development of effective methods for treating neurodegenerative diseases, stroke and traumatic brain injury. Indeed, numerous studies have shown that ischemic stroke induces the production of new neurons in the damaged striatum from the SVZ. However, the potential for plasticity of these cells after brain injury remains a highly controversial issue (Kernie & Parent, 2010). Furthermore, little is known about the origins and genetic profiles of these newborn neurons in the damaged striatum after brain injury. Thus, there has been a gap in our understanding of normal neural development and ectopic neurogenesis after brain injury. In this study, we have taken advantage of genetic fate mapping to permanently label newly born neurons in the ischemic damaged striatum to address this issue.

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cells in the dorsal–lateral SVZ only give rise to CR+ interneurons in the damaged striatum. As in rat brains, nearly all newborn young and mature neurons in the mouse striatum continuously express the transcription factor Sp8. By contrast, no newborn neuroblasts differentiate into medium spiny neurons, the primary striatal projection neurons. Surprisingly, when Sp8 was conditionally inactivated in the Emx1-expressing lineage, a subpopulation of CR+ cells in the normal and damaged striatum ectopically expressed Pax6. Moreover, these cells had large cell bodies and long processes. The present study identifies the origin of the newly born CR+ interneurons in the damaged striatum after ischemic brain injury. This also indicates that Emx1-expressing neural stem cells might be a valuable manipulation target for brain repair after injury.

Materials and methods

Mice

C57BL/6 mice were obtained from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). Z/EG (Novak et al., 2000) and Emx1-Cre (Gorski et al., 2002) mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Sp8 conditional mutant mice were obtained from crossing double heterozygous males (Emx1-Cre; Sp8flx/flx) with Sp8 homozygous flox (Sp8Flox/flox) females (Waclaw et al., 2006). All experiments using animals were carried out in accordance with institutional guidelines, and the study was approved by the Fudan University Animal Care and Use Committee. All efforts were made to minimize the number of animals used.

Induction of perinatal H/I injury

Perinatal H/I injury was produced in 8-day-old mice (day of birth was designated P0) by a permanent unilateral common carotid artery ligation followed by systemic hypoxia. Briefly, pups were lightly anesthetized with isoflurane (4% induction, 2% maintenance). Once fully anesthetized, a midline neck incision was made and the right common carotid artery was identified. The right common carotid artery was separated from the vagus nerve and then ligated with 4-0 silk. Pups were returned to the dam for 2 h. Before being exposed to hypoxia (8% O2/92% N2), the pups were prewarmed for 10 min in jars submerged in a 37 °C water bath. The pups were then exposed to hypoxia for 50 min. After hypoxia the pups were returned to the dam and maintained under standard conditions.

BrdU injections

BrdU (50 mg/kg; Sigma, St. Louis, MO, USA) was injected intraperitoneally (i.p.) twice daily (8-h intervals) during days 3–6 after H/I injury (in total, 4 days eight times). Animals were perfused 3, 5 or 13 weeks after H/I injury.

Immunohistochemistry

Mice were given intracardiac perfusion of 4% paraformaldehyde and 0.5% glutaraldehyde, and postfixed overnight. Fifty-micron sections were cut on the vibratome and washed in TBS three times for 10 min. Sections were then blocked for 1 h in 10% donkey serum and incubated for 48 h at 4 °C with anti-Sp8. Secondary antibody (biotinylated donkey-anti goat) was incubated for 3 h at room temperature. Sections were then incubated with Streptavidin-coupled horseradish peroxidase for 1 h and revealed with dianinobenzidine (DAB). After immunostaining the sections were post-fixed in 2% osmium for 2 h, rinsed, dehydrated and embedded in Araldite. To recognize the SVZ, 1-μm semithin sections were stained with 1% toluidine blue. To further identify individual cells in the SVZ, we cut 60-nm ultrathin sections with a diamond knife. The sections were stained with lead citrate and uranyl acetate, and examined under a Philips CM120 electron microscope.

Electron microscopy

For Sp8 pre-embedding immunostaining, adult mice were perfused with 4% paraformaldehyde and 0.5% glutaraldehyde, and postfixed overnight. Fifty-micron sections were cut on the vibratome and washed in TBS three times for 10 min. Sections were then blocked for 1 h in 10% donkey serum and incubated for 48 h at 4 °C with anti-Sp8. Secondary antibody (biotinylated donkey-anti goat) was incubated for 3 h at room temperature. Sections were then incubated with Streptavidin-coupled horseradish peroxidase for 1 h and revealed with dianinobenzidine (DAB). After immunostaining the sections were post-fixed in 2% osmium for 2 h, rinsed, dehydrated and embedded in Araldite. To recognize the SVZ, 1-μm semithin sections were stained with 1% toluidine blue. To further identify individual cells in the SVZ, we cut 60-nm ultrathin sections with a diamond knife. The sections were stained with lead citrate and uranyl acetate, and examined under a Philips CM120 electron microscope.
TABLE 1. Primary antibodies used in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunogen</th>
<th>Source, catalogue no., species, dilution</th>
<th>Specificity and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrdU</td>
<td>BrdU</td>
<td>Accurate Chemical, OBT0030, rat monoclonal IgG2a, 1 : 500</td>
<td>No staining was observed in cases in which animals were not infused with BrdU</td>
</tr>
<tr>
<td>Calretinin</td>
<td>Recombinant rat calretinin</td>
<td>Millipore, AB1550, goat polyclonal, 1 : 2000</td>
<td>WB recognizes both the calcium-bound and calcium-unbound conformations of calretinin (data sheet). IHC pattern was identical to that previously described (Yang et al., 2008)</td>
</tr>
<tr>
<td>Calretinin</td>
<td>Recombinant rat calretinin</td>
<td>Millipore, AB5054, rabbit antiserum, 1 : 4000</td>
<td>WB recognizes both the calcium-bound and calcium-unbound conformations of calretinin (data sheet). IHC pattern was identical to that previously described (Yang et al., 2008)</td>
</tr>
<tr>
<td>ChAT</td>
<td>ChAT purified from rat brain</td>
<td>Millipore, MAB305, mouse monoclonal IgG1, 1 : 100</td>
<td>WB (data sheet); IHC pattern was identical to that previously described (Yang et al., 2008)</td>
</tr>
<tr>
<td>DARPP-32</td>
<td>Peptide: CVEMIRRRRPTPAML surrounding Thr34 of human DARPP-32</td>
<td>Cell Signaling, no. 2302, rabbit polyclonal, 1 : 200</td>
<td>WB (data sheet); IHC pattern was identical to that previously described (Yang et al., 2008)</td>
</tr>
<tr>
<td>Doublecortin</td>
<td>Peptide: amino acids 385–402 of human Dcx</td>
<td>Santa Cruz, sc-8066, goat polyclonal, 1 : 100</td>
<td>WB and IHC pattern consistent with previous studies (Brown et al., 2003; Suzuki et al., 2007)</td>
</tr>
<tr>
<td>Doublecortin</td>
<td>Peptide: amino acids 300 to the C terminus of human Dcx</td>
<td>Abcam, Ab-18723, rabbit polyclonal, 1 : 1,000</td>
<td>WB (data sheet); IHC pattern consistent with previous work (Liu et al., 2009)</td>
</tr>
<tr>
<td>Foxp1</td>
<td>Peptide: amino acids 650 to the C terminus of human Foxp1</td>
<td>Abcam, ab16645, rabbit polyclonal, 1 : 500</td>
<td>WB (data sheet); IHC pattern consistent with previous work (Liu et al., 2009)</td>
</tr>
<tr>
<td>Green fluorescent protein</td>
<td>Recombinant GFP</td>
<td>Aves Labs, GFP-1020, chicken polyclonal, IgY, 1 : 2000</td>
<td>WB (data sheet); selective staining of GFP-expressing cells</td>
</tr>
<tr>
<td>NeuN</td>
<td>Mouse brain cell nuclei</td>
<td>Millipore, MAB377, mouse monoclonal IgG1, 1 : 300</td>
<td>WB (data sheet); IHC pattern consistent with previous work (Mullen et al., 1992)</td>
</tr>
<tr>
<td>Parvalbumin</td>
<td>Parvalbumin purified from frog muscle</td>
<td>Millipore, MAB1572, mouse monoclonal IgG1, 1 : 1000</td>
<td>By immunoblot it recognizes a single band at 12 kDa (data sheet). The staining pattern was identical to that previously described (Yang et al., 2008)</td>
</tr>
<tr>
<td>Pax6</td>
<td>Peptide: amino acids 420–436 of mouse Pax6</td>
<td>Covance, PRB-278P, rabbit polyclonal, 1 : 300</td>
<td>WB (data sheet). The pattern of staining was similar to that previously described (Brill et al., 2009)</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Peptide: amino acids 1–106 of human somatostatin</td>
<td>Santa Cruz, sc-13099, rabbit polyclonal, 1 : 100</td>
<td>WB (data sheet). The pattern of staining was similar to that previously described (Yang et al., 2008)</td>
</tr>
<tr>
<td>Sp8</td>
<td>Peptide: amino acids 491–508 of human Sp8</td>
<td>Santa Cruz, sc-104661, goat polyclonal, 1 : 500</td>
<td>WB (data sheet). The pattern of staining was similar to that previously described (Waclaw et al., 2006; Liu et al., 2009)</td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry; WB, Western blot.

using FV10-ASW software. The number presented was the mean number of cells per section.

CR+ cells were counted with an epifluorescence microscope using a ×40 objective. Six to eight sections at 180-μm intervals (every six sections) of each brain were quantified (n = 4 mice each group). Briefly, in each section, the entire striatal cross-section was scanned on the right and left, and all CR+ cells in the striatum were visualized by focusing up and down. Cells were counted if they contained a whole cell body. The number presented was the mean number of cells per section.

Dcx+ or Dcx+/Sp8+ cells in the normal SVZ were quantified with confocal microscopy (n = 3 mice). We selected six sections in each brain. Confocal Z sectioning was performed at 0.5-μm intervals in each triple labeling section (Dcx+/Sp8+/DAPI) using a ×60 objective. Cells were counted from individual optical sections, not collapsed projection images. About 300–700 cells were analysed in each region of the SVZ.

To measure the longest axis and the cell body area of CR+, CR+/Sp8+, CR+/Pax6+ and CR+/Sp8+EGFP cells in the striatum of Emx1-Cre; Z/EG mice or Emx1-Cre; Sp8EGFP/Δmice, confocal Z-sectioning was first performed at 1.0-μm intervals in the section using a ×60 (NA = 1.42) objective. The data was then obtained using Neurolucida.

**Statistical analysis**

All values are presented as means ± SEM. We used Student’s paired t-test, or one-way ANOVA followed by Fisher’s post-hoc LSD test with SAS software to analyse numeric data. We considered P values < 0.05 as statistically significant.

**Results**

**Newly born neurons in the ischemic injured striatum are CR+/Sp8+ interneurons**

Doublecortin (Dcx) is a microtubule-associated protein that is expressed primarily in neurally committed precursors and immature

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neurons. Thus, Dcx+ cells are always located in the neurogenic regions, such as the SVZ and hippocampal dentate gyrus, and the expression of Dcx can be used to identify young neurons newly derived from neural stem/progenitor cells. Dcx+ cells were rare within the striatum of the intact (contralateral) or normal control brain at postnatal week 4 (P28) or later (Fig. 1A). In contrast, an increase in Dcx+ cells was readily observed in the damaged (ipsilateral) striatum from 3 to 13 weeks after the induction of perinatal H/I injury in P8 mice. Nearly all of these cells expressed Sp8 (Fig. 1B–E). In some cases, Dcx+ cells were not observed to migrate into the neocortex and these cells also expressed Sp8 (Fig. S1A). Interestingly, the vast majority of Dcx+ cells in the walls of the lateral ventricle, including the dorsal–lateral, lateral, medial and ventral SVZ, express Sp8, regardless of whether the brain is damaged (Figs 1 and 2) (Waclaw et al., 2006). This phenomenon was further confirmed by whole-mount Dcx/Sp8 double immunostaining and electron microscopy (Fig. 2G–I). To test whether this is the rule or an exception, we also examined Sp8 expression in an adult mouse stroke model. Two weeks after stroke, we observed that Dcx+ cells in the damaged striatum exclusively express Sp8 (Fig. S1B). The above observations suggest that Dcx+ cells do not change their intrinsic properties after ischemic injury as they continuously express Sp8 during their migration from the SVZ to the damaged striatum of mice.

To label newly generated cells following H/I injury, the S-phase marker BrdU was injected intraperitoneally for 4 days beginning at day 3 after the induction of H/I injury and BrdU/Dcx double immunostaining was performed after 3 weeks of recovery. In the damaged areas of the ipsilateral striatum, many BrdU+/Dcx+ cells were found and they possessed the morphologies characteristic of migrating neuroblasts (Fig. S2A–E). Quantitative analysis revealed a significant increase in the number of BrdU+/Dcx+ cells in the ipsilateral striatum compared with the contralateral striatum (84 vs. three cells per section, Fig. S2F). We also observed that some of the Dcx+ cells in the damaged striatum expressed CR at 3 weeks recovery from H/I injury, but none of these cells expressed Foxp1 (Fig. S3), a transcription factor expressed by the medium spiny neurons in the striatum (Tamura et al., 2004).

To follow the fate of newborn Dcx+ cells, mice were killed 5 weeks after H/I injury. As anticipated, a subpopulation of mature neurons, BrdU+/NeuN+ cells that coexpressed CR, were found in the damaged striatum (approximately 2–3 cells per section, Fig. S4A). By contrast, we never observed any other striatal neurons that were generated in the damaged striatum after H/I injury, such as DARPP-32+ (a specific marker for medium spiny neurons), parvalbumin+, somatostatin+ or ChAT+ cells (Fig. S4B–E). Intriguingly, there was also a very small number of BrdU+/NeuN+ cells in the damaged areas of the striatum that did not express any of the striatal or olfactory bulb (OB) neuronal markers (Fig. S4F). The phenotypes of these neurons remain to be determined. We noted that all BrdU+/CR+ cells in the damaged striatum continued to express Sp8 (Fig. S4G and H). Consistent with this observation, more CR+/Sp8+ cells in the damaged striatum were observed compared with the contralateral striatum 5 weeks after H/I injury (Fig. 3). Together, these results indicate that while newborn neuroblasts are plentiful in the ischemic injured striatum, only a few can differentiate into CR+/Sp8+ interneurons. This is consistent with our previous observations on adult rats following stroke (Liu et al., 2009). These studies also suggest that there are no major species-specific differences in the properties of the newborn neurons in the damaged striatum of the murine brain following ischemic injury.

![Fig. 1. Dcx+ cells in the SVZ and damaged striatum express Sp8.](image-url)
CR+ interneurons in the ischemic injured striatum are derived from the Emx1-expressing neural stem cells in the dorsal–lateral SVZ.

Because neural stem cells in the SVZ are heterogeneous with respect to their embryonic origins (Young et al., 2007), we wanted to know which domain of the SVZ gives rise to new CR+ cells in the damaged striatum after H/I injury. Given previous results that neural stem cells in the Emx1 domain but not in the lateral ganglionic eminence contribute to more than 40% CR+ interneurons in the OB (Kohwi et al., 2007), we hypothesized that Emx1-expressing stem cells may also produce new CR+ cells in the damaged striatum. We crossed the Emx1-Cre mouse line to cre-reporter line, Z/EG mice (Novak et al.,...
that permanently express enhanced GFP (EGFP) in cells from the Emx1-expressing lineage (Gorski et al., 2002).

In the normal striatum, the absolute number of CR+ cells is very low (Rymar et al., 2004). However, these cells originate from different brain germinal regions such as the medial ganglionic eminence and Emx1 expression domain (Marin et al., 2000; Willaime-Morawek & van der Kooy, 2008). We observed that most of the CR+ cells in the intact striatum express Sp8 (Fig. 3A, C and E). In the adult Emx1-Cre; Z/EG mice, CR+ cells in the striatum can be divided into three subgroups (Fig. 4). Group 1, the CR+/Sp8+/EGFP+, cells are mainly located in the striatum adjacent to the dorsal–lateral SVZ, which constitutes 80% of CR+ cells in the striatum (Fig. 4A, D and E). The second group of cells, comprising 10% of striatal CR+ cells, are CR+/Sp8+ but EGFP-immunonegative, mainly locating in the striatum adjacent to ventral SVZ (Fig. 4B, D and E). The third group, which makes up 10% of the total striatal CR+ cells and is interspersed within the whole striatum, is CR+ but Sp8+/EGFP double-immunonegative (Fig. 4C–E) and may be derived from Nkx2.1+ progenitor cells in the medial ganglionic eminence (Marin et al., 2000). The morphologies of these cells are different, as CR+/Sp8+ cells are smaller (Fig. 4A–C′, F).

In agreement with the results of wild type C57BL/6 mice, more CR+ cells were readily observed in the damaged striatum compared with the control or contralateral striatum of Emx1-Cre; Z/EG mice (Fig. 5A and B). CR/EGFP double immunostaining combined with analysis of single confocal optical sections revealed that, surprisingly, more than 90% of CR+ cells (258 of 275, n = 3) in the damaged striatum expressed EGFP (Fig. 5A and B). This indicates that most, if not all, newly born CR+ interneurons in the ischemic injury striatum are derived from the Emx1-expressing neural stem cells. Again, no BrdU+/DARPP32+/EGFP+ cells were found in the damaged striatum following H/I injury.

In the embryonic neuroepithelium, Emx1-expressing neural stem cells could be found in multiple regions, including both pallium and subpallium (Gorski et al., 2002; Willaime-Morawek et al., 2006; Cocos et al., 2009; Waclaw et al., 2009). However, in the SVZ of Emx1-Cre; Z/EG mice at P8 or later, EGFP+/Sp8+ cells were mainly located in the dorsal–lateral region (areas around the pallial–subpallial boundary), but were rare in the lateral and ventral regions (data not shown). Moreover, Dcx+/EGFP+ cells appeared to migrate from the dorsal–lateral SVZ into the damaged areas of the ipsilateral striatum after perinatal H/I injury (Fig. 5C–E). In contrast, Dcx+ cells seldom expressed EGFP if these cells migrated into the damaged striatum from the lateral or ventral SVZ (Fig. 5D). Thus, we propose that Emx1-expressing neural stem cells in the dorsal–lateral SVZ contribute to most of the newly born CR+ interneurons in the damaged striatum after perinatal H/I injury.

Conditional inactivation of Sp8 alters morphogenesis and Pax6 expression in a subpopulation of CR+ interneurons in the normal and damaged striatum

Previous studies have indicated that Sp8 contributes to the generation of a large subpopulation of OB interneurons. To investigate the role of Sp8 in the production of CR+ interneurons in the normal and damaged striatum, we inactivated Sp8 in the Emx1-expressing lineage by using Cre/LoxP recombination. Emx1-Cre; Sp8FloRev/−
double heterozygous males were crossed with Sp8\textsuperscript{Flux/\textsuperscript{flox}} females (Waclaw et al., 2006) to produce Sp8 conditional mutants: Emx1-Cre; Sp8\textsuperscript{Flux/\textsuperscript{flox}} mice. With the exception of noticeably smaller OBs, all of these Sp8 conditional mutants were indistinguishable from their wild-type and Emx1-Cre; Sp8\textsuperscript{Flox/\textsuperscript{flox}} littermates from postnatal to adult stage. As expected, there was a visible decrease in Sp8 expression in the dorsal–lateral SVZ and rostral migratory stream (RMS) of Sp8 conditional mutants (Fig. 6A–C). By contrast, more Pax6\textsuperscript{+} cells were found in the dorsal–lateral SVZ and RMS (Fig. 6E–G). In the OB, similarly, Pax6 expression was increased while Sp8 expression was decreased (data not shown). These results are consistent with previous reports by using Dlx5/6-CIE; Sp8\textsuperscript{Flux/\textsuperscript{flox}} conditional mutant mice (Waclaw et al., 2006).

In the normal striatum of adult Emx1-Cre; Sp8\textsuperscript{Flux/\textsuperscript{flox}} conditional mutants, we did not detect Sp8 expression in most CR\textsuperscript{+} cells (Fig. 6I and J). This observation further supports the notion that the Emx1-expressing lineage contributes to most CR\textsuperscript{+} interneurons in the striatum. Unexpectedly, we also found that a subpopulation (about 15%) of CR\textsuperscript{+} cells ectopically expressed Pax6 in the striatum of Sp8 conditional mutants (Fig. 6K and L; 81 of 553, \(n = 4\) mice), whereas CR\textsuperscript{+}/Pax6\textsuperscript{+} cells were never found in the striatum of wild-type and Emx1-Cre; Sp8\textsuperscript{Flox/\textsuperscript{flox}} mice. Interestingly, the morphology of CR\textsuperscript{+}/Pax6\textsuperscript{+} cells was different from that of CR\textsuperscript{+} cells, which did not express Pax6 in the striatum of Sp8 conditional mutants, as the cell body was significantly larger (64.1 ± 3.8 vs. 33.9 ± 1.6 \(\mu\)m, Student’s t-test, \(P < 0.001\)) and the longest process was significantly longer (124.2 ± 12.6 vs. 43.2 ± 3.8 \(\mu\)m, Student’s t-test, \(P < 0.001\)).

As in the wild-type and Emx1-Cre; Sp8\textsuperscript{Flux/\textsuperscript{flox}} mice, in the ischemic damaged striatum of adult Emx1-Cre; Sp8\textsuperscript{Flux/\textsuperscript{flox}} mice, we found that there were noticeably more Dcx\textsuperscript{+} cells; many of them did not express Sp8 (Fig. 7A). Accordingly, compared with the contralateral striatum, significantly more CR\textsuperscript{+} cells in the damaged striatum of Sp8 conditional mutants were found (Fig. 7B and E). Similarly, Sp8 expression was not detected in most of these CR\textsuperscript{+} cells (Fig. 7B), and a subpopulation of CR\textsuperscript{+} cells with large cell bodies and longer processes expressed Pax6 (Fig. 7C). Occasionally, a few BrdU\textsuperscript{+}/CR\textsuperscript{+}/Pax6\textsuperscript{+} cells were found in the damaged striatum after perinatal H/1 injury, suggesting they were newly generated (Fig. 7D).

It is worth noting that whereas most CR\textsuperscript{+} cells in the normal and damaged striatum of Sp8 conditional mutant mice did not express Sp8, the numbers of CR\textsuperscript{+} cells were not significantly different from those in the wild-type and Emx1-Cre; Sp8\textsuperscript{Flux/\textsuperscript{flox}} mice, respectively (Fig. 7E). Thus, migration of Dcx\textsuperscript{+} neuroblasts and maturation of CR\textsuperscript{+} interneurons in the normal and damaged striatum seemed unaffected by conditional inactivation of Sp8.

Discussion

In the present study, we found that: (i) most CR\textsuperscript{+} interneurons in the normal striatum express the transcription factor Sp8; (ii) as in rat brains, there are significantly more newborn neuroblasts and mature CR\textsuperscript{+} interneurons that express Sp8 in the ischemic damaged striatum compared with the contralateral striatum of mouse brains; (iii) the Emx1-expressing lineage contributes to most of the CR\textsuperscript{+} interneurons in the normal and ischemic damaged striatum; (iv) conditional inactivation of Sp8 in the Emx1-expressing lineage results in a subpopulation of CR\textsuperscript{+} cells in the striatum ectopically expressing Pax6; and (v) CR\textsuperscript{+}/Pax6\textsuperscript{+} cells in the striatum possess large cell bodies and long processes.
It has become generally accepted that neural stem cells in the normal SVZ, the largest germinal region of the postnatal brain, mainly give rise to interneurons and some glial cells, but not large projection neurons (Luskin, 1993; Lois & Alvarez-Buylla, 1994; Young et al., 2007; Batista-Brito et al., 2008; Yang, 2008). Besides OB interneurons, even in the undamaged brain, SVZ neural stem cells can give rise to some CR+ interneurons in the striatum of perinatal mouse brain and adult rat brain (Dayer et al., 2005; Inta et al., 2008). In the damaged brain, however, whether neural stem cells in the SVZ are able to generate projection neurons remains controversial (Magavi et al., 2000; Arvidsson et al., 2002; Nakatomi et al., 2002; Parent et al., 2002; Teramoto et al., 2003; Cho et al., 2007; Yang et al., 2008; Brill et al., 2009; Liu et al., 2009; Kernie & Parent, 2010). During development, neural stem cells in different regions of the nervous system give rise to different subtypes of neurons, oligodendrocytes or astrocytes (Kessaris et al., 2006; Hochstim et al., 2008; Kriegstein & Alvarez-Buylla, 2009), suggesting that they are very diverse. Moreover, neural stem/progenitor cells are resistant to respecification by changing their environment (Desai & McConnell, 2000; Carletti et al., 2002; Mukouyama et al., 2006; Shen et al., 2006; Merkle et al., 2007; Rolando et al., 2010). Supporting and complementing this interpretation, our work further clearly shows that even in the damaged brain, the fate of Emx1-lineage cells in the striatum is very restricted, because they do not give rise to any other subtypes of neurons except CR+ interneurons.

Recent studies have identified that the neurogenic niche of the postnatal SVZ is more extensive than previously thought (Kohwi et al., 2007; Merkle et al., 2007; Ventura & Goldman, 2007; Young et al., 2007). Neural stem cells in the postnatal SVZ are actually derived from radial glial cells in the different domains of the embryonic forebrain, including septum, cortex, and lateral and medial ganglionic eminence (Kriegstein & Alvarez-Buylla, 2009). Among
these stem/progenitor cells, Emx1-lineage cells are unique as they sequentially generate the extraordinary diversity of neuronal types in the brain. Indeed, Emx1-lineage cells contribute to nearly all cortical projection neurons and a subpopulation of striatal projection neurons during early embryonic development (Gorski et al., 2002; Willaime-Morawek et al., 2006; Cocas et al., 2009). They also produce various subtypes of interneurons in late embryonic and early postnatal development. Even in the adult brain, Emx1-expressing neural stem cells in the SVZ continuously give rise to several different subpopulations of interneurons in the OB (Kohwi et al., 2007; Young et al., 2007). Currently, we do not know whether single Emx1-expressing neural stem cells in vivo have this dual or multiple potential, or whether separate subpopulations of Emx1-expressing neural stem cells that generate projection neurons or interneurons coexist in the germinal VZ/SVZ of the brain. We also do not know why so many CR+ interneurons derived from the Emx1-expressing lineage were produced in the ischemic injured striatum.

Previous studies and our recent report demonstrated that the vast majority of neuroblasts in the SVZ–RMS–OB system of adult murine brain express Sp8 (Waclaw et al., 2006; Liu et al., 2009). Most mature interneurons in the adult OB continuously express Sp8 as well
(Waclaw et al., 2006; Liu et al., 2009). A similar phenomenon occurs in the majority of neuroblasts in the SVZ–RMS–OB system and mature OB interneurons in the adult rhesus monkey brain (C. Wang and Z. Yang, unpublished observations). Although previous work has already indicated that Sp8 contributes to the survival, migration and molecular specification of a subpopulation of CR+ OB interneurons (Waclaw et al., 2006), additional functions of Sp8 in the SVZ–RMS–OB system need to be investigated.

In the present study, we found that there were significantly more newborn Dcx+ neuroblasts and CR+ interneurons in the damaged striatum than the contralateral striatum of both wild-type and Sp8 conditional mutants. Therefore, it seems that conditional inactivation of Sp8 in the Emx1-expressing lineage does not affect neuroblast migration and CR+ cell maturation in the normal and damaged striatum. When Sp8 was conditionally inactivated in the Emx1-expressing lineage, many neuroblasts in the SVZ and RMS no longer expressed Sp8. By contrast, only a few neuroblasts started to ectopically express Pax6. Accordingly, in the normal and damaged striatum, only about 15% CR+ cells expressed Pax6. This suggests that even Emx1-derived Sp8+ neuroblasts in the SVZ and Emx1-derived Sp8+/CR+ interneurons in the normal and damaged striatum are heterogeneous. In addition, we found that ectopic expression of Pax6 in a subpopulation of CR+ cells in the striatum is correlated with their morphological changes. If Emx1-derived CR+ cells in conditional mutant striatum do not express Pax6, their morphology is similar to that of CR+/Sp8+ cells in the striatum of wild-type mice. However, when a subpopulation of CR+ cells in the striatum of conditional mutants ectopically express Pax6, their cell body becomes larger and their processes become longer. The phenotype of these CR+ cells is never observed in the striatum of wild-type mice. Thus, inactivation of Sp8 and ectopic activation of Pax6 are sufficient to change the morphology of some CR+ cells in the striatum. How Sp8 and Pax6 co-regulate these CR+ cells in the striatum to acquire specific morphology remains unknown. Our results also suggest that although the Emx1-expressing lineage is highly patterned, as with other neural stem cells in the SVZ and their progenies in the brain, this does not mean that neural stem cells are irreversibly committed in vivo. Future efforts to understand the molecular mechanisms of fate specification of neural stem cells and their progenies may help us to develop an effective stem cell-based therapeutic approach for various neurological disorders.

Supporting Information

Additional supporting information may be found in the online version of this article:
Fig. S1. Dcx+ cells in the SVZ, ischemic injured striatum and cortex express Sp8.
Fig. S2. New Dcx+ cells are generated in the damaged striatum after H/I injury.
Fig. S3. Some of Dcx+ cells in the damaged striatum express CR, but none of them expresses Foxp1.
Fig. S4. The types of newborn neurons in the ischemic injured striatum are very limited.

Fig. 7. In the Sp8 conditional mutant mice, there are more Dcx+ and CR+ cells in the damaged striatum. (A, B) Many Dcx+ cells (A) and the majority of CR+ cells (B) do not express Sp8. (C) Some CR+ cells express Pax6 (arrows) in the damaged striatum. (D1–2) Occasionally, BrdU+/CR+/Pax6+ cells (arrow) can be found in the damaged striatum of Sp8 conditional mutants. Note that the cell body of the CR+ but Pax6-negative cell (arrowhead) is smaller than that of the BrdU+/CR+/Pax6+ cell (arrow). (E) Quantitative data revealing that there are significantly more CR+ cells in the damaged striatum than the contralateral striatum in both wild-type and transgenic mice, respectively. *P < 0.05, one-way ANOVA followed by Fisher’s post-hoc LSD test, n = 4 mice per group. Scale bars = 50 μm (A–C) and 10 μm (D1–2).


Yang, Z. (2008) Postnatal subventricular zone progenitors give rise not only to granular and periglomerular interneurons but also to interneurons in the external plexiform layer of the rat olfactory bulb. *J Comp Neurol.*, 506, 347–358.
