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Patterns and dynamics of subventricular zone neuroblast migration in the ischemic striatum of the adult mouse

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Abstract

The migratory behavior of neuroblasts after a stroke is poorly understood. Using time-lapse microscopy, we imaged migration of neuroblasts and cerebral vessels in living brain slices of adult doublecortin (DCX, a marker of neuroblasts) enhanced green fluorescent protein (eGFP) transgenic mice that were subjected to 7 days of stroke. Our results show that neuroblasts originating in the subventricular zone (SVZ) of adult mouse brain laterally migrated in chains or individually to reach the ischemic striatum. The chains were initially formed at the border between the SVZ and the striatum by neuroblasts in the SVZ and then extended to the striatum. The average speed of DCX-eGFP-expressing cells within chains was 28.67±1.04 μm/h, which was significantly faster ($P < 0.01$) than the speed of the cells in the SVZ (17.98±0.57 μm/h). Within the ischemic striatum, individual neuroblasts actively extended or retracted their processes, suggestive of probing the immediate microenvironment. The neuroblasts close to cerebral blood vessels exhibited multiple processes. Our data suggest that neuroblasts actively interact with the microenvironment to reach the ischemic striatum by multiple migratory routes.

Keywords

chain migration; doublecortin (DCX); enhanced green fluorescent protein (eGFP); neuroblast; stroke; subventricular zone (SVZ)

Introduction

The subventricular zone (SVZ) of the lateral ventricles of the adult rodent contains neural stem cells, transient amplifying progenitor cells, and neuroblasts (Alvarez-Buylla and Garcia-Verdugo, 2002; Alvarez-Buylla and Lim, 2004). Neural stem cells produce transient amplifying cells that generate neuroblasts (Doetsch *et al*, 1997; Alvarez-Buylla and Garcia-Verdugo, 2002; Alvarez-Buylla and Lim, 2004). Neuroblasts express doublecortin (DCX), a protein that regulates neuroblast migration (Yang *et al*, 2004; Nam *et al*, 2007; Walker *et al*, 2007). Under physiologic conditions, these neuroblasts travel the rostral migratory stream (RMS), as chains through tunnels formed by astrocytes or use blood vessels as a scaffold, to

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the olfactory bulb where they differentiate into granule and periglomerular neurons throughout the adult life (Doetsch *et al*, 1997; Luskin *et al*, 1997; Garcia-Verdugo *et al*, 1998; Bovetti *et al*, 2007).

Focal cerebral ischemia induces neurogenesis in the SVZ (Zhang *et al*, 2001,2004*a*;Arvidsson *et al*, 2002;Parent *et al*, 2002;Jin *et al*, 2003). Neuroblasts generated in the SVZ migrate toward the ischemic boundary, and neuroblasts are closely associated with cerebral vessels (Zhang *et al*, 2004*a*;Yamashita *et al*, 2006;Thored *et al*, 2007). Within the ischemic boundary, some neuroblasts mature into interneurons to replenish damaged neurons (Lindvall *et al*, 2004;Zhang *et al*, 2004*a*;Yamashita *et al*, 2006). Thus, migration of the neuroblasts from the SVZ to the ischemic boundary is a critical step in the repair of the injured brain. However, dynamic migratory patterns of the neuroblast population in the ischemic brain have not been investigated, although a few studies have examined motility of DCX and nestin-positive cells in the olfactory bulb under normal and ischemic conditions (Nam *et al*, 2007;Zhao and Nam, 2007). In this study, using a transgenic mouse line that expresses DCX-enhanced green fluorescent protein (eGFP) (Walker *et al*, 2007) and time-lapse microscopy, we imaged the patterns of DCX-eGFP cell movement in acute adult brain slices after stroke. Our findings indicate that neuroblasts originating in the SVZ take multiple migratory routes to reach the ischemic boundary.

Materials and methods

All experiments procedures were approved by the Institutional Animal Care and Use Committee of Henry Ford Hospital.

DCX-eGFP Transgenic Mice

A pair of breeding colony DCX-eGFP transgenic mice (DCX-GFP/bacterial artificial chromosome (BAC)) was purchased from the Mutant Mouse Regional Resource Center, Davis, CA, USA. Adult DCX-eGFP animals (3 months old) were identified by PCR analysis using tail DNA with primers to GFP (450 bp): forward 5′-TGCAGTGCTTCAGCCGCTACC-3′; reverse 5′-ATGTGATCGCGCTTCTCGTTG-3′.

Animal Model of Middle Cerebral Artery Occlusion

Male DCX-eGFP mice (3 months old) were subjected to permanent focal cerebral ischemia by inserting a 6-0 nylon filament (9 to 9.5 mm in length) into the right middle cerebral artery (MCA) (Zhang *et al*, 1997*b*). Occlusion of the right MCA for 7 days induces the ischemic lesion in the territory supplied by the MCA (Supplementary Figure 1) and evokes a peak increase of neurogenesis (Zhang *et al*, 2001, 2004*a*). Therefore, all mice were killed 7 days after MCA occlusion. Nonischemic DCX-eGFP mice (3 months old) were used as a control group.

Organotypic Brain Slice Culture

Nonischemic mice (n= 4) and 7-day ischemic mice (n=9) were killed. The procedure used for the organotypic slice culture assay is based on published protocols (Miyata *et al*, 2002; Zhang *et al*, 2007). When removed from the skull, the brain was immediately immersed in ice-cold Hank's balanced salt solution (Gibco, Carlsbad, CA, USA) at pH 7.2 for 3 mins. A 4-mm-thick coronal brain section between the level of bregma (2.7 mm) and the bregma (-1.3 mm) was used for the organotypic brain slice culture (Franklin and Paxinos, 1997), which includes the entire territory supplied by the MCA (Zhang *et al*, 1997*a*). The coronal brain section was cut into 300-μm-thick sections using a vibratome, and the coronal slices were placed in a six-well plate with one slice per well. In some cases, sagittal sections at the striatal SVZ plane were cut. Brain tissue was kept in ice-cold Hank's balanced salt solution during these procedures. The

brain slice was then embedded in the Cellmatrix gel (200 μl, Type 1-A Nitta Gelatin, Osaka, Japan) and incubated for 1 h with the brain slice media containing Dulbecco's modified Eagle's medium/F-12, insulin (25 μg/mL), APO-transferrin (100 μg/mL), progesterone (20 nmol/L), sodium selenate (30 nmol/L), putrescine (60 μmol/L), epidermal growth factor (10 ng/mL), basic fibroblast growth factor (10 ng/mL), horse serum (5%), fetal calf serum (5%), penicillin —streptomycin (1%), sodium bicarbonate (1%), and HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 0.33%) (Miyata *et al*, 2002). Thereafter, the brain slices were ready for time-lapse microscopy studies.

Time-Lapse Microscopy

Organotypic brain slices were incubated in a stage top chamber with 5% CO_2 and 37°C (LiveCell Control Unit), which was placed on the stage of a Nikon TE2000-U inverted microscope (Nikon, Tokyo, Japan) equipped with a motorized Z-stage. A × 10 objective with 1.5× electronic zoom was used for taking images. Fluorescent field images were taken for the brain slices with excitation and emission filters at 546 and 585 nm, respectively, at 0.5 msecs exposure times. A stack of images (36 images with a 5-μm step in Z axes) was acquired at 15-min intervals during 4 to 10 h using a CCD camera (CoolSnap, Tuscon, AZ, USA) and MetaView Software (Universal Imaging, Dowiningtown, PA, USA).

Quantification of Cell Motility

Cell migration speed and distances, and lengths of cell processes were analyzed offline by tracing individual cells with the best focus plane from a Z-stack at different times using Universal Imaging MetaMorph Software. Subsequently, migratory values were statistically analyzed.

Immunohistochemistry

To examine whether DCX-eGFP-expressing cells are DCX positive in the transgenic mouse, nonischemic mice (n=4) and mice subjected to 7 days of stroke (n = 6) were killed and their brains were fixed for immunostaining. Immunostaining for fixed brain coronal sections were carried out according to a published protocol (Zhang *et al*, 2004*b*; Robin *et al*, 2006). The following primary antibodies were used in this study: rabbit anti-glial fibrillary acidic protein (1:500; Dako Cytomation California, Carpinteria, CA, USA), goat anti-DCX (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and chicken anti-GPF (1:500, Aves Labs, Tigard, OR, USA). Double immunofluorescent images were taken using a Zeiss confocal microscope (Zeiss LSM 510 NLO, Thornwood, NY, USA).

Statistical Analysis

The measurements of migration distance and speed were presented as mean±s.e. Significant differences between the SVZ and chains were analyzed using Student's t-test. Statistical significance was set at $P < 0.05$.

Results

Stroke Increases DCX-eGFP-Expressing Cells in the Ischemic Boundary

On the basis of immunohistochemistry, with an antibody against DCX in fixed brain tissue, we and others have shown that stroke increases neuroblasts in the ischemic SVZ and the ischemic boundary (Zhang *et al*, 2001,2004*a*;Arvidsson *et al*, 2002;Parent *et al*, 2002;Jin *et al*, 2003). To examine whether DCX-eGFP-expressing cells are DCX positive in the transgenic mouse, we performed double immunostaining with antibodies against GFP and DCX. In the nonischemic mouse, GFP-positive cells were detected only in the SVZ of the lateral ventricles on coronal brain sections, and all GFP-positive cells were DCX positive (Figures 1A to 1D).

After stroke, GFP-positive cells increased in the ipsilateral SVZ. Some GFP-positive cells formed a chain-like structure extending from the SVZ to the striatal ischemic boundary, and these GFP-positive cells were closely associated with cerebral vessels (Figure 1E). In addition, GFP-positive cells localized to the ischemic striatum exhibited bipolar morphology with a long leading process or formed a cluster around the cerebral vessels (Figures 1F to 1L). Double immunostaining showed that GFP-positive cells after stroke were DCX immunoreactive (Figures 1F to 1K), but glial fibrillary acidic protein (a marker of astrocytes) was negative (Figure 1M). Distribution and morphology of GFP-positive cells in the ischemic brain of the transgenic mouse are consistent with the published DCX data (Arvidsson *et al*, 2002;Parent *et al*, 2002;Jin *et al*, 2003;Zhang *et al*, 2004*a*).

Neuroblasts Migrate Laterally out of the Subventricular Zone in Chains or Individually to Reach the Ischemic Boundary

Aforementioned results indicate that the transgene of DCX-eGFP reflects the endogenous DCX gene expression under normal and ischemic conditions. Therefore, we used this transgenic mouse line to examine the migratory patterns of neuroblasts in living brain slices. Brain coronal slices at the striatal level were obtained from nonischemic mice (n=4) and mice subjected 7 days of middle cerebral artery occlusion (n = 9). Dynamic movement of DCX-eGFP-expressing cells was imaged using time-lapse microscopy. In nonischemic brain slices, DCX-eGFP-expressing cells in the dorsolateral and ventrolateral SVZs of the lateral ventricles exhibited round shape morphology with a short process (23±1.0 μm, n = 176 cells on 20 brain slices from 4 mice, Figure 2B, arrows). The cell body of DCX-eGFP-expressing cells actively migrated dorsally and ventrally along the lateral ventricular surface and did not migrate laterally into the striatum on the coronal sections (Supplementary Movie 1), which is consistent with earlier studies on SVZ cell motility (Suzuki and Goldman, 2003; Nam *et al*, 2007). However, in ischemic brain coronal slices, some DCX-eGFP-expressing cells in the dorsal and ventral SVZs migrated laterally in chains to reach the ischemic striatum (Figure 2A). Thus, at least two concurrent migratory patterns of DCX-eGFP-expressing cells were observed: DCX-eGFP-expressing cells within the SVZ moved vertically to the surface of the lateral ventricles (Supplementary Movie 2a), whereas DCX-eGFP-expressing cells in chains migrated horizontally from the lateral ventricles toward the ischemic striatum (Figures 2C to 2E and Supplementary Movie 2b). Chain migration of DCX-eGFP-expressing cells to the ischemic striatum was also observed in brain saggital sections including the striatal SVZ (Supplementary Figure 2), suggesting that the chain migration of DCX-eGFP-expressing cells to the ischemic striatum is unlikely caused by interrupting the RMS after coronal sectioning (Alonso *et al*, 1999; Kirschenbaum *et al*, 1999). To start the formation of a chain, a DCX-eGFP-expressing cell within a SVZ cluster extended its leading process pointing to the ischemic striatum and then moved out of the cluster to the border between the SVZ and the striatum (Figure 2F, a white arrow). Individual DCX-eGFP-expressing cells within the SVZ cluster consequently emigrated to follow the first DCX-eGFP-expressing cells (Figure 2F, a red arrow). These cells actively moved around the SVZ border and the ischemic striatum by retracting or extending their processes, suggesting that they are searching for a special microenvironment (Figure 2F, white and red arrows). Eventually, these cells formed a chain that extended from the SVZ to the ischemic striatum (Figure 2F white, red, and blue arrows, Supplementary Movie 3). Once chains were formed, individual DCX-eGFP-expressing cells in the SVZ continually migrated to the chains. When a DCX-eGFP-expressing cell within the SVZ started to migrate to a chain, it extended a long leading process from the cell body with the tip of the leading process directed to the chain (Figure 2G). Then, this DCX-eGFP-expressing cell moved to the base of the chain and emigrated from the SVZ to join the chain (Figure 2G, white arrow). Within the chain, DCX-eGFP-expressing cells were highly motile, rapidly extending or retracting the leading process and migrating latero-medially (Supplementary Movie 4). A chain was temporarily disrupted when a DCX-eGFP-expressing cell moved medially to the SVZ (Figure 2H,

arrowhead). However, the chain was reformed when the DCX-eGFP-expressing cell moved laterally to the ischemic striatum by extending its leading process following the original path of the chain (Figure 2H, arrows). The orientation of the chain migration did not change during the experimental period (10 h), suggesting that chains are stable. An average length of migrating chains measured from the border of the SVZ of the lateral ventricle to the ischemic striatum was 452.3±32.5 μm in the dorsal SVZ (total 82 chains from 9 mice), which was significantly longer ($P < 0.05$) than the length in the ventral SVZ (304.7±34.9 μm from 48 chains of 9 mice). Occasionally, a DCX-eGFP-expressing cell within a chain moved out of the chain and extended a leading process toward its neighbor chain (Figure 2I, white arrow). Subsequently, this cell moved into the neighboring chain with nuclear translocation (Figure 2I, white arrow). Motile DCX-eGFP-expressing cells were defined by a speed faster than 9 μm/h, as it was difficult to measure the total distance moved over several hours for the cells with the speed < 9 μm/h in this study. With this characterization, we found that 71.7%±3.23% ($n = 112$ cells of total 156 cells measured) and 31.3%±1.27% ($n = 152$ cells of total 486 cells measured) of DCX-eGFP-expressing cells within chains and the SVZ, respectively, were migrating. The average speed of motile DCX-eGFP-expressing cells within chains was 28.67±1.04 μm/h, which was significantly faster ($P < 0.01$) than the speed of the cells in the SVZ (17.98±0.57 μm/h, Table 1). In addition to chain migration, we also observed that individual DCX-eGFP-expressing cells migrated laterally from the SVZ to reach the ischemic striatum. Migration of DCX-eGFP-expressing cells to the ischemic cortex was never observed during the time-lapse experimental period.

Movement of Neuroblasts within the Ischemic Striatum

Within the ischemic striatum, individual DCX-eGFP-expressing cells migrated latero-medially or dorsoventrally (Figures 3C and 3D). DCX-eGFP-expressing cells in the ischemic striatum had a much longer leading process (62.9±1.56 μm, $n = 355$ cells) compared with those within the SVZ (22.5±1.01 μm, $n = 424$ cells, $P < 0.05$). The cell body always moved to the direction that the leading process pointed (Figure 3C). During movement, DCX-eGFP-expressing cells actively changed their directions by extending and retracting their leading process, possibly, to explore the immediate microenvironment (Figures 3B and 3D, Supplementary Movie 5). We found that 39%±2.2% (93 cells of total 240 cells measured) of DCX-eGFP cells extended a new leading process during movement. Before a DCX-eGFP-expressing cell changed its direction, a second branch from the leading process was generated (Figure 3D, white arrowhead). The cell then moved to a new direction pointed to by the new branch (Figure 3D, white arrowhead) and the original branch was retracted (Figure 3D, white arrow). A few DCX-eGFP-expressing cells moved to the direction that was opposite to its original direction by giving rise to a new leading process at the opposite pole of the cell body (Figure 3D, red arrowhead). Sometimes, individual DCX-eGFP-expressing cells from different positions pointed their processes to the same direction and then correspondingly moved their nuclei (Supplementary Figure 3). However, when the leading processes extended closely to each other, one cell moved away from that direction by retracting its original process and extended a new leading process (Supplementary Figure 3). Studies from fixed brain tissue and *in vitro* neurosphere assay suggest that neuroblasts divide during migration (Zhang *et al*, 2007). Within the ischemic striatum, we also observed a few dividing DCX-eGFP-expressing cells (Figure 3E, white arrow). A DCX-eGFP-expressing cell retracted its process during division and then divided into two DCX-eGFP-expressing daughter cells (Figure 3E, white arrows). One daughter cell extended a leading process and moved away from the other (Figure 3E, white arrows).

Neuroblast Migration is Closely Associated with Cerebral Vessels in the Ischemic Striatum

Our immunostaining data from fixed brain tissue showed that DCX-eGFP-positive cells formed a cluster around cerebral vessels (Figure 1E). To examine whether neuroblasts use cerebral

vessels as a scaffold to reach the ischemic boundary, cerebral vessels were perfused with rhodamine-dextran in the living mouse (Zhang *et al*, 1999). Ten minutes after the injection of rhodamine-dextran, the animal was killed and the brain slices were harvested. Using different wavelengths, DCX-eGFP-expressing cells (green) and cerebral vessels filled with rhodaminedextran (red) were simultaneously imaged by means of time-lapse microscopy. We found that a few of the migratory chains were parallel to cerebral vessels and that DCX-eGFP-expressing cells at the end of the chain migrated out of the chain to vessels and formed a cluster nearby vessels (Figure 4B, red arrowheads). Within the ischemic striatum, individual DCX-eGFP-expressing cells with a leading process moved along vessels (Figure 4B, white arrow). We also observed that DCX-eGFP cells migrated from the SVZ to reach cerebral vessels in the ischemic striatum (Figure 4C). A single DCX-eGFP-expressing cell in the SVZ first extended its leading process directed to a cerebral blood vessel and then migrated to the vessel (Figure 4C, white arrow). When it reached the vessel, the DCX-eGFP-expressing cell retracted its leading process and joined other DCX-eGFP-expressing cells that already presented adjacent to the vessel. These cells formed a cluster and actively moved around the vessels (Figure 4C, Supplementary Movie 6). In contrast to the individual DCX-eGFP-expressing cells distant from blood vessels, the DCX-eGFP-expressing cells adjacent to blood vessels exhibited multiple branches and their movement seemed saltatory over several hours, although their processes actively changed (Figure 4C, Supplementary Movie 6). These data suggest that cerebral blood vessels are associated with neuroblast migration (Bovetti *et al*, 2007).

Discussion

Histologic analysis of fixed brain specimens suggests that stroke redirects neuroblasts in the SVZ to the ischemic striatum (Arvidsson *et al*, 2002; Parent *et al*, 2002; Jin *et al*, 2003; Zhang *et al*, 2003, 2004*a*). The migratory behavior of neuroblasts after stroke is poorly understood. Using time-lapse imaging of the DCX-eGFP transgenic mouse line, we provide direct evidence to show that neuroblasts originating in the SVZ of the adult mouse brain migrate in chains or individually to reach the ischemic striatum. During formation of a chain and a migration, DCX-eGFP-expressing cells actively change their movement directions by extending and retracting their leading processes. These data suggest that neuroblasts actively interact with microenvironment to reach the ischemic striatum by multiple migratory routes.

Under physiologic conditions, neuroblasts in the SVZ of the lateral ventricles migrate as chains in the RMS to the olfactory bulb where they differentiate into granule and periglomerular neurons throughout the adult life (Doetsch *et al*, 1997; Luskin *et al*, 1997; Garcia-Verdugo *et al*, 1998; Bovetti *et al*, 2007). This study provides, for the first time, patterns and dynamics of SVZ neuroblast migration in the ischemic brain of the adult mouse. Consistent with earlier findings, under nonischemic conditions, neuroblasts migrated dorsa-ventrally within the SVZ of the lateral ventricle and never crossed the SVZ striatum border (Suzuki and Goldman, 2003; Nam *et al*, 2007; Zhang *et al*, 2007). However, after stroke, many neuroblasts emigrated laterally from the SVZ to reach the ischemic striatum by either chains or individual migration, suggesting that neuroblasts cross some structural and/molecular barriers between the SVZ and the adjacent striatum that constrains neuroblast migration under physiologic conditions (Suzuki and Goldman, 2003). These findings show that when crossing these barriers, neuroblasts seem to exhibit exploratory behavior, to the sample microenvironment cues between the SVZ and the adjacent striatum. These exploratory cells then migrated back to be in contact with other neuroblasts in the SVZ and with them form a chain following the path mapped by the exploratory neuroblasts. These data suggest that after stroke, neuroblasts actively communicate with each other during chain formation and that microenvironmental cues are important for directing neuroblast migration. Thus, migration of neuroblasts to the ischemic striatum is likely mediated by intrinsic determinants in neuroblasts and extrinsic factors generated by the ischemic lesion. Using a neurosphere assay, we showed earlier that stroke alters intrinsic the

SVZ cell migratory behavior that is independent of the brain microenvironment (Zhang *et al*, 2007). In addition to a marker of migrating neuroblasts, DCX plays an essential role in maintaining bipolar morphology of neuroblasts (Koizumi *et al*, 2006; Ocbina *et al*, 2006; Friocourt *et al*, 2007; Walker *et al*, 2007). Consistent with the published data, we observed that the majority of DCX-eGFP-expressing cells exhibited bipolar morphology with a long leading process in the ischemic brain, and these cells often changed their movement direction by extending another branch and retracting its original process. Collectively, DCX could be one of the potential intrinsic proteins that regulate neuroblast migration in the ischemic brain.

Chain migration is a feature of SVZ progenitor cells (Wichterle *et al*, 1997). In this study, we observed that neuroblasts used chain migration to reach the ischemic striatum, suggesting that neuroblasts maintain their characteristics under ischemic conditions. The chains appear as individual tunnels from the SVZ to the ischemic striatum, and neuroblasts within a chain migrate one following the other. These findings are consistent with chain-like structure of neuroblasts in the ischemic striatum observed in fixed tissue (Figure 1) (Zhang *et al*, 2004*a*), suggesting that this kind of chain migration is likely present *in vivo*. Chains were actively disrupted and reformed as a result of a bidirectional movement of individual neuroblasts, although the orientation of chains did not change. These data may explain why fewer chains are found in fixed brain tissue, which only captures a single time point. Interestingly, individual neuroblasts always followed the original chain path to re-assemble the chain, suggesting that some molecules in the ischemic striatum restrict neuroblast migration within a chain. Individual chains spanned from the dorsal and ventral SVZs to directionally reach the ischemic striatum with different lengths. Neuroblasts within chains had a much faster speed than the cells in the SVZ. In contrast, individual neuroblasts within the ischemic striatum exhibited nondirectional movement by frequently changing their movement directions, indicative of a complex microenvironment of the ischemic striatum. We therefore speculate that in response to a stroke, neuroblasts use directional chain migration to rapidly cross the complex microenvironment of the striatum to reach the ischemic boundary, which permits delivery of a large number of neuroblasts to repair the ischemic brain (Wichterle *et al*, 1997). It is unlikely that these individual migration chains are ensheathed by astrocytes because such a chain architecture of astrocytes has not been reported in the ischemic brain. Furthermore, studies *in vitro* show that neuroblasts can assemble into chains and migrate to each other without the assistance of the ensheathing astrocytes (Wichterle *et al*, 1997). It will be important to identify molecules that restrict the neuroblast migration to the chain path in the ischemic brain.

Migration speed was measured based on the two-dimensional images, which is inherently inaccurate compared with a three-dimensional analysis by underestimating migration speed. Interestingly, using two-photon microscopy, Zhao and Nam (2007) show that an average speed of nestin-GFP-expressing cells in the RMS is 30 μm/h measured by three-dimensional calculation in 3-week-old nestin-GFP mice, which is comparable with an average speed of 29 μm/h observed in DCX-eGFP-expressing cells within a chain.

This study shows that a selective neuroblast population in the SVZ participates in chain migration directed to the ischemic striatum. Neuroblasts in the SVZ are a heterogeneous population derived from different neural stem cells (Willaime-Morawek *et al*, 2006; Merkle *et al*, 2007; Ventura and Goldman, 2007; Young *et al*, 2007). Stroke induces changes in gene profiles of neuroblasts localized to the SVZ (Liu *et al*, 2009). Whether neuroblasts emigrating laterally from the SVZ to the ischemic striatum comprise a subgroup of neuroblasts remains to be determined.

Neural stem cells and transit amplifying cells lie adjacent to the SVZ vascular plexus, whereas neuroblasts in the SVZ use cerebral vessels as a scaffold to migrate to the olfactory bulb (Bovetti *et al*, 2007; Shen *et al*, 2008; Tavazoie *et al*, 2008). This study showed that individual

neuroblasts migrate to blood vessels and form a multicell cluster around blood vessels. These data further support that angiogenesis and neurogenesis are coupled in the ischemic brain (Lee *et al*, 2006; Ohab *et al*, 2006; Robin *et al*, 2006; Wang *et al*, 2006; Teng *et al*, 2008). Cerebral endothelial cells activated by stroke secrete stromal-derived factor 1α, a CXC chemokine, vascular endothelial growth factor, Ang1, and matrix metalloproteinases (Lee *et al*, 2006; Ohab *et al*, 2006; Robin *et al*, 2006; Wang *et al*, 2006; Teng *et al*, 2008). These molecules likely act as extrinsic factors generated by the ischemic lesion to attract neuroblasts to the ischemic lesion (Lee *et al*, 2006; Ohab *et al*, 2006; Robin *et al*, 2006; Wang *et al*, 2006; Teng *et al*, 2008). In addition, activated cerebral endothelial cells secrete brain-derived growth factor that is known to promote neurite outgrowth (Leventhal *et al*, 1999). Stroke induces neuroblasts to express mature neuronal marker genes (Liu, 2009). These data along with the present observation that neuroblasts adjacent to vessels exhibit multiple processes resembling more mature neurons suggest that angiogenic vessels could promote maturation of neuroblasts. The molecules responsible for tethering neuroblasts to a vascular niche in the ischemic brain are not known. A recent study shows that blockage of the laminin receptor, α6*β*1 integrin, causes SVZ neural stem and progenitor cells to move away from cerebral blood vessels (Shen *et al*, 2008). Laminin expression is upregulated in angiogenic vessels (Fujita *et al*, 2006). These findings raise the possibility that α6*β*1 integrin—laminin interactions could be important to tether neuroblasts to cerebral blood vessels. One of the limitations of this study is that we were not able to detect all cerebral microvessels perfused by rhodamine-dextran in brain slices, which could contribute to our observation that only a few chain migrations were closely associated blood vessels. Thus, it is not known whether cerebral blood vessels guide construction of chain migration in the ischemic striatum.

In summary, this study provides direct evidence that neuroblasts in the SVZ primarily use chain migration to reach the ischemic striatum and that cerebral blood vessels and neuroblast migration are coupled. These data serve as a base to further investigate molecular mechanisms underlying neuroblast migration after stroke. Therapies that target amplifying neuroblast chain migration and angiogenic vessels may enhance neurogenesis in the ischemic brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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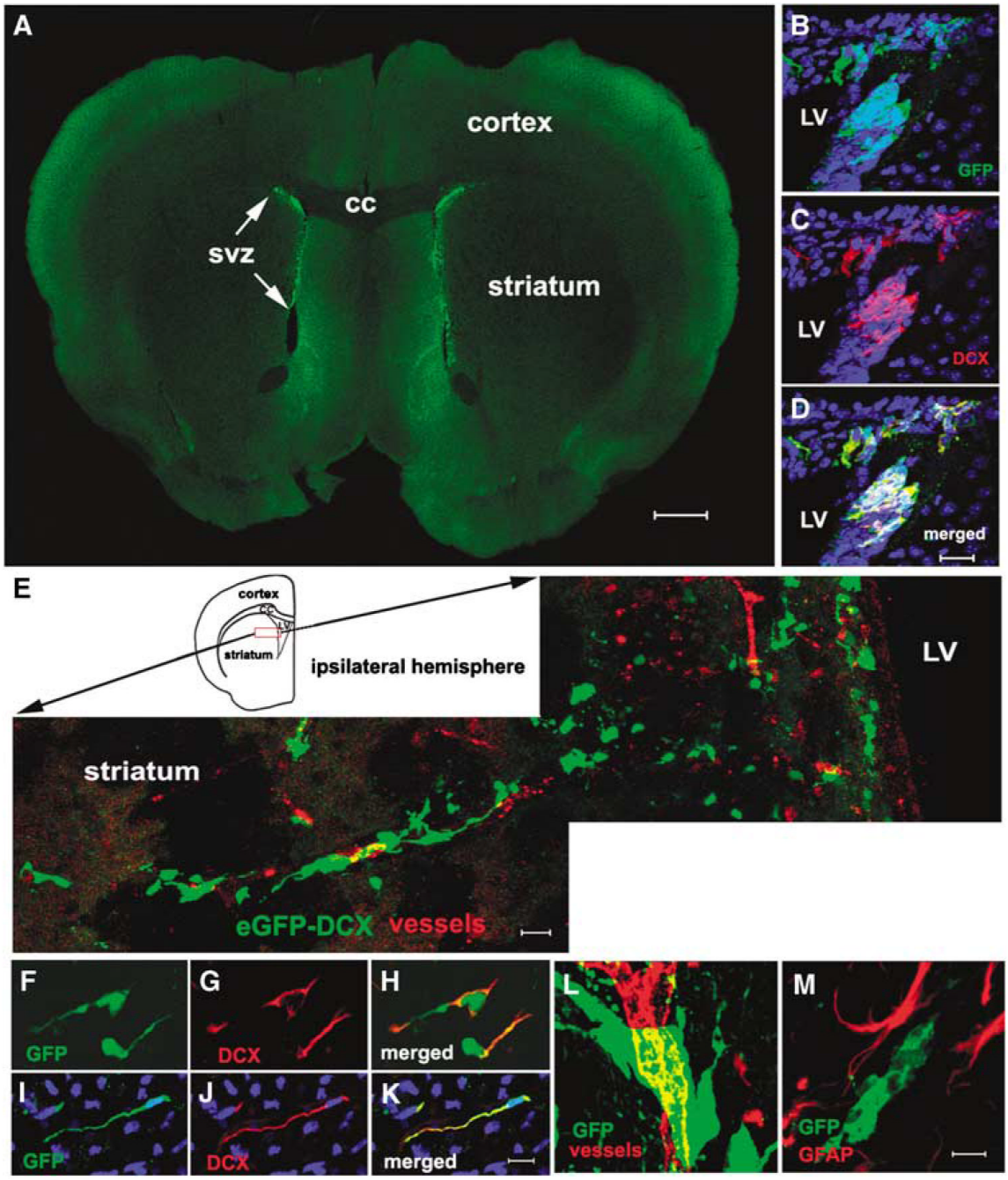

Figure 1.

DCX-eGFP expression in normal and ischemic brains of the adult DCX-eGFP transgenic mice. All images were acquired from fixed brain tissue. A coronal section at the striatal level of a normal brain shows that DCX-eGFP-positive cells only localize to the SVZ of the lateral ventricles (**A**, white arrows). Double immunostaining from a representative normal mouse shows that GFP-positive cells in the SVZ (**B** and **D**) are DCX immunoreactive (**C** and **D**). A microphotograph from a representative mouse subjected to 7 days of stroke shows a chain migration of DCX-eGFP-positive cells from the SVZ to the ischemic striatum (**E**, green), which was closely associated with cerebral blood vessels (**E**, red). A red box in diagram of the ipsilateral hemisphere at the striatal level of the coronal section indicates where the image was

taken (**E**). Within the ischemic striatum, DCX-eGFP-positive cells exhibited bipolar morphology with long leading processes (**F**, **H**, **I**, and **K**) and these cells were DCX positive (**G**, **H**, **J**, and **K**). Some DCX-eGFP-positive cells (**L**, green) surrounded blood vessels (**L**, red). DCX-eGFP-positive cells (**M**, green) were not glial fibrillary acidic protein (GFAP) positive (**M**, red). Scale bars = 1 mm in panel A; 20 μm in panels D and E; and 10 μm in panels K and M. Blue color, cell nuclei; CC, corpus callosum; and LV, lateral ventricle.

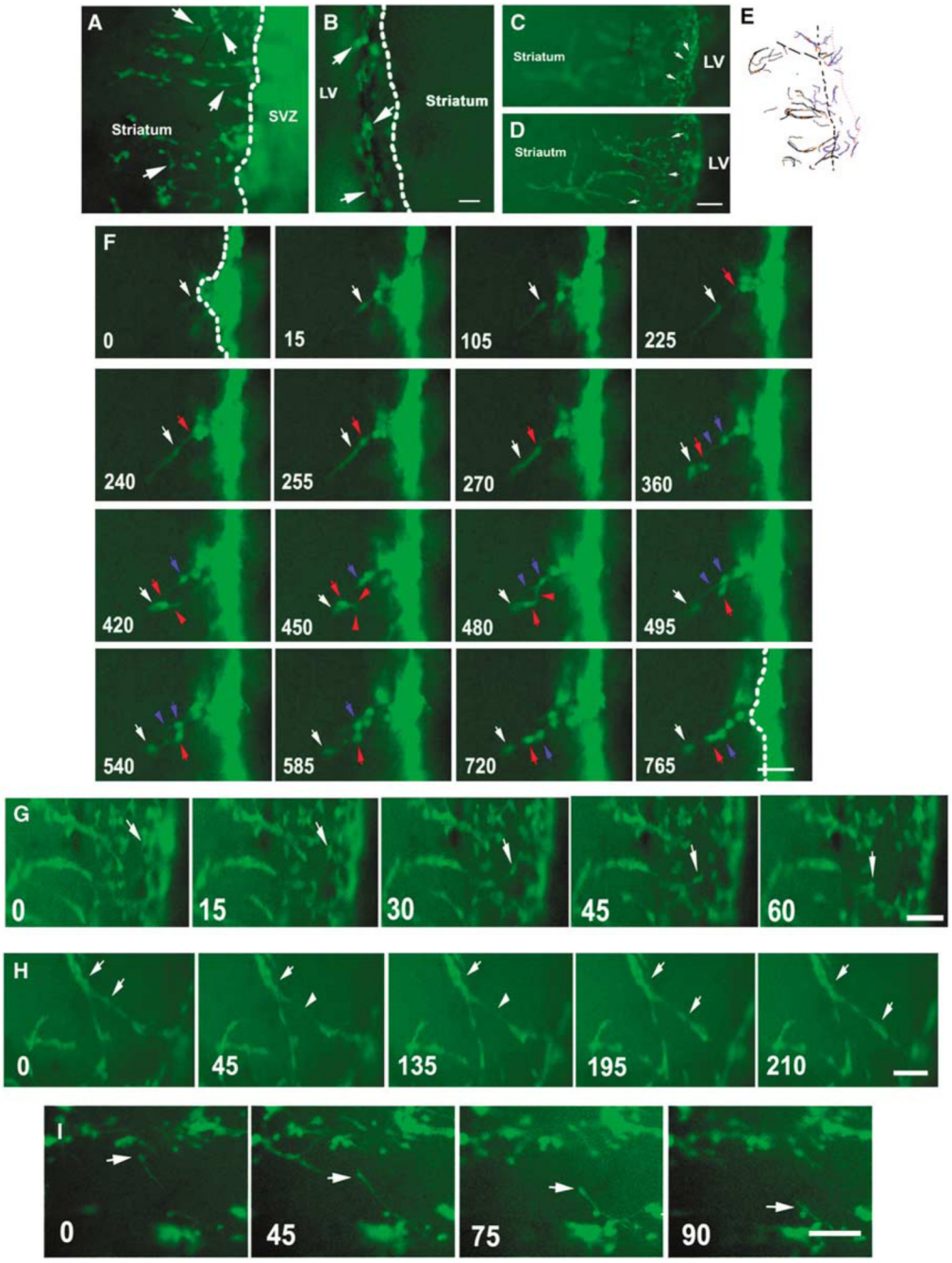

Figure 2.

Time-lapse imaging of DCX-eGFP-expressing cells in living brain slices obtained from the normal and ischemic mice. Coronal brain slices at the striatal level show that DCX-eGFP-expressing cells emigrated laterally from the SVZ to the ischemic striatum in chains (**A**) compared with the DCX-eGFP-expressing cells within the SVZ of the nonischemic mouse (**B**). DCX-eGFP-expressing cells in the SVZ exhibited short processes and moved dorsally and ventrally along the lateral ventricular surface (**C**, arrows), whereas DCX-eGFP-expressing cells in chains showed bipolar morphology with long leading processes and moved laterally from the SVZ in an ischemic brain slice (**D**, arrows). Images of C and D were acquired from the same brain slice at different focus. Computer tracking movement paths of individual DCX-

eGFP-expressing in panels C and D shows the presence of dorso-ventral (black lines, 25 cells) and lateral-medial migration (blue lines, 30 cells) paths in the SVZ and the striatum, respectively (**E**). (**F**) Time-lapse imaging of DCX-eGFP-expressing cells to form a chain that crossed the border between the SVZ and the adjacent striatum (a dash line) in an ischemic brain slice. Arrows in each frame of panel F point out the location of the cell body, whereas arrowheads indicate the leading process. Each color represents individual DCX-eGFP-expressing cells (also see Supplementary Movie 3). (**G**) A selective DCX-eGFP-expressing cell migrating from the SVZ to a chain in an ischemic brain slice. An arrow in each frame of panel G points out the location of the cell body. (**H**) Disruption and reformation of a chain structure composed by DCX-eGFP-expressing cells in an ischemic brain slice. Arrows in each frame point out a connection of a chain, whereas an arrowhead indicates the location of a disrupted chain. (**I**) A DCX-eGFP-expressing cell moving out of a chain to join an adjacent chain in an ischemic brain slice. An arrow in each frame points out the location of the cell body. Numbers in panels F to I indicate time in minutes. Scale bars = 50 μm.

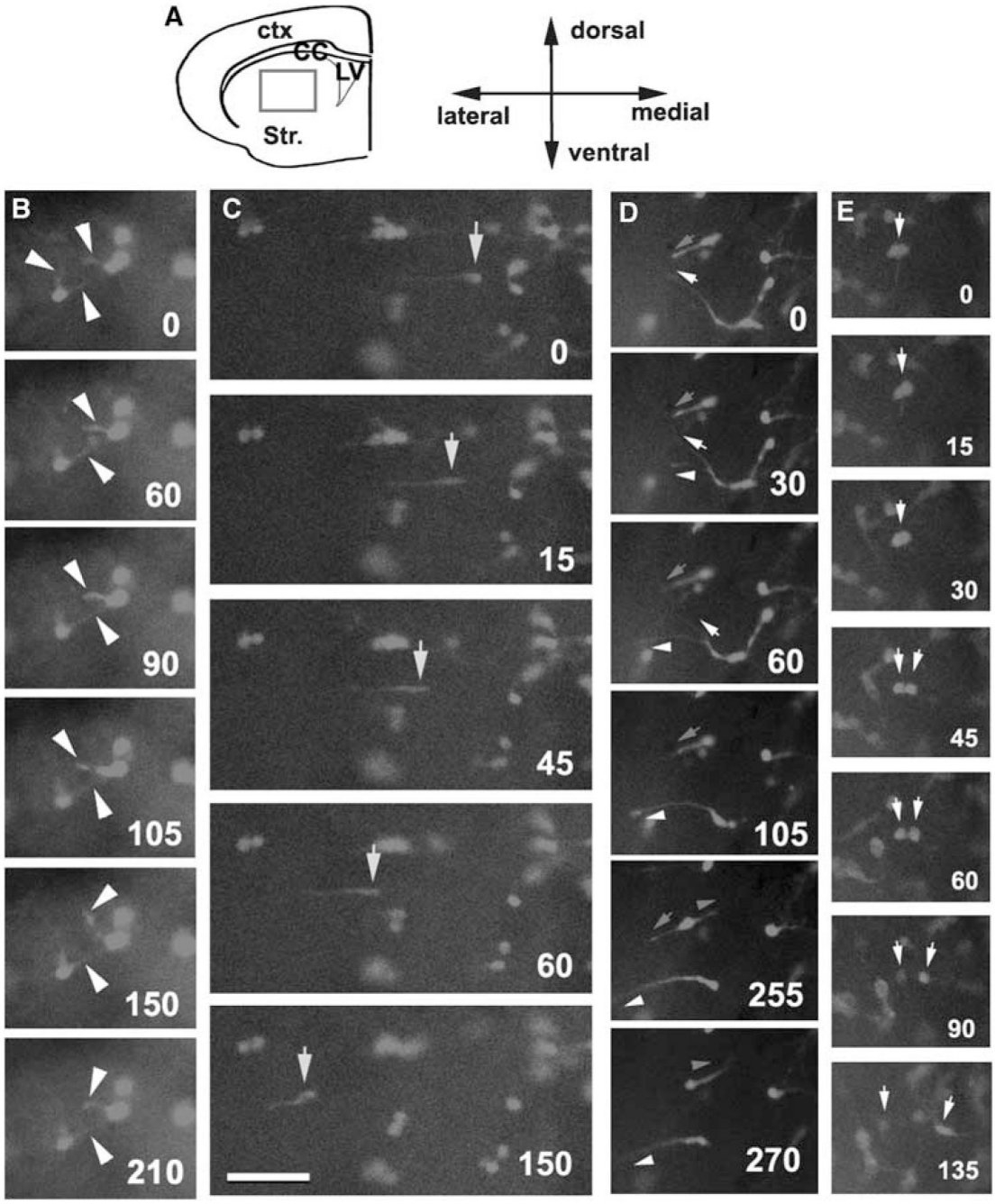

Figure 3.

Time-lapse imaging of DCX-eGFP-expressing cells in the ischemic striatum of living brain slices obtained from the ischemic transgenic mice. A red box in the diagram of the ipsilateral hemisphere indicates where the images in this figure were acquired (**A**). (**B**) The cytoplasmic processes (arrowheads) of individual DCX-eGFP-expressing cells actively changed directions by extending and retracting to explore the immediate microenvironment. (**C**) A cell body (arrow) moved to the direction where the leading process pointed. An arrow in each frame of panel C points out the location of the cell body. (**D**) Two DCX-eGFP-expressing cells changed their original movement directions (white and red arrows) by sending a second branch from the exited process (a white arrowhead) or producing a new process at the opposite side of the

cell body (a red arrowhead). (**E**) A DCX-eGFP-expressing cell divided into two daughter cells and arrows indicate location of the cell bodies. Numbers in panels B to E indicate time in minutes. Scale bar = 50 μm.

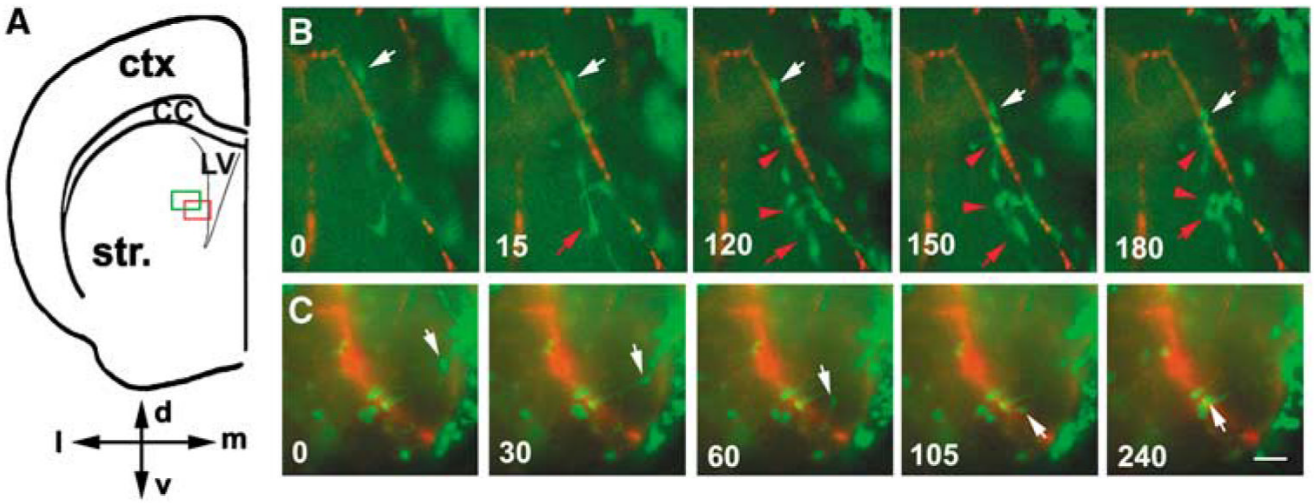

Figure 4.

Time-lapse imaging of DCX-eGFP-expressing cells and cerebral blood vessels in the ischemic SVZ and the striatum of living brain slices obtained from the ischemic transgenic mice. Green and red boxes in the diagram of the ipsilateral hemisphere indicate where the images in panels B and C, respectively, were acquired (**A**). (**B**) An individual DCX-eGFP-expressing cell (a white arrow) within the ischemic striatum moved along a blood vessel (red), whereas DCX-eGFP-expressing cells in a chain (a red arrow) emigrated from the chain to form a cluster adjacent to a blood vessel (red arrowheads). (**C**) A DCX-eGFP-expressing cell emigrated from the SVZ (arrow) to a cerebral blood vessel (red) in the ischemic striatum. Green color in panels B and C indicates DCX-eGFP-expressing cells; red color in panels B and C indicates cerebral blood vessels. Numbers in panels B and C indicate time in minutes. Scale bar = 50 μm.

Table 1

Movement rate of cell in the ischemic brain

Locations	Migratory speed (μm/h)		
	Average	Maximum	Minimum
SVZ (*n* = 152 cells)	17.9±0.58	42.54	9.7
Chain (*n* = 112 cells)	28.7±1.04	54.37	9.8
Striatum (*n* = 104 cells)	37.1±1.71	81.3	14.63

SVZ, subventricular zone.

Mean±s.e.