Published in final edited form as:

Exp Neurol. 2013 October ; 248: 246–253. doi:10.1016/j.expneurol.2013.06.017.

Stromal derived growth factor-1 (CXCL12) modulates synaptic transmission to immature neurons during post-ischemic cerebral repair

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Abstract

In response to ischemic injury, the brain mounts a repair process involving the development of new neurons, oligodendrocytes, and astrocytes. However, the manner in which new neurons integrate into existing brain circuitry is not well understood. Here we observed that during the four weeks after transient middle cerebral artery occlusion (MCAO), doublecortin (DCX)-expressing neural progenitors originating in the subventricular zone (SVZ) were present in the ischemic lesion borderzone, where they received γ -aminobutyric acid (GABA) inputs, a feature that is common to newly developing neurons. The chemokine stromal derived factor-1 (SDF-1 or CXCL12) was enriched in lesional endothelial and microglial cells for up to four weeks after transient MCAO, and application of SDF-1 to acute brain slices enhanced GABAergic inputs to the new neurons. These observations suggest that SDF-1 is in a position to coordinate neovascularization and neurogenesis during the repair process after cerebral ischemia-reperfusion.

Keywords

Animal models; brain ischemia; chemokines; electrophysiology; neuroregeneration

INTRODUCTION

Stroke is the fourth leading cause of death in the United States behind heart disease, cancer, and chronic lung disease and is a leading cause of disability world-wide (Roger, et al., 2010). Annually, more than 750,000 Americans experience strokes, the majority of which are ischemic. Acute treatment with intravenous tissue plasminogen activator results in improved outcome in ischemic stroke, but its narrow therapeutic time window of 3 hours from symptom onset (as approved by the Federal Drug Administration, or 4.5 hours when used off-label) severely limits real-world applicability. As a result, the majority of patients

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who live through an ischemic stroke survive with neurologic disability which may limit activities of daily living including ambulation.

The brain is able to repair itself to some extent: injury sets in motion pathophysiologic processes, some of which have reparative functions and may result in improvement of neurologic function. Injury-related processes include angiogenesis, neurogenesis, inflammation, and gliogenesis: stem cells from several different regions give rise to neural precursors in the aftermath of brain injury in adult animals (Ohab and Carmichael, 2008). In the case of ischemia of the middle cerebral artery (MCA) territory, the SVZ, which normally gives rise to the rostral migratory stream, becomes the source of neural precursors, some of which change course and migrate toward the lesion (Zhang, et al., 2008). These newly generated cells, some of which are neuroblasts by virtue of their expression of DCX, target the perilesional region, an area where processes related to neural repair and plasticity take place. The question of whether these new cells are able to stably and durably integrate and augment functional recovery is currently under investigation, as are potential pharmacologic agents that may enhance their functional integration.

The neurotransmitter GABA influences maturation of neural stem cells during the development of the nervous system, as well as in the SVZ and subgranular zone (SGZ) of the hippocampus in the adult. GABAergic inputs to neural stem cells in the dentate gyrus (DG) develop before glutamatergic mediated inputs (Ben-Ari and Holmes, 2005, Bordey, 2007, Ge, et al., 2006, Tozuka, et al., 2005), and neural stem cells are modulated by excitatory GABAergic synaptic inputs during early development (Ge, et al., 2007). GABA is also critical for cortical plasticity and sensory mapping: altering GABAergic transmission changes sensory maps during the critical period of cortical development (Hensch, 2005). Cortical GABAergic signaling through GABA_A receptors is divided into synaptic (phasic) and extrasynaptic (tonic) components. The tonically active extrasynaptic GABA_A receptors set an excitability threshold for neurons during normal development and after ischemic stroke (Clarkson, et al., 2010, Glykys and Mody, 2006, Walker and Semyanov, 2008).

One class of molecules that may have important effects on reparative processes including GABAergic signaling is the chemokine family. Although chemokines have been thought to function primarily in systemic immunologic surveillance and host defense, several chemokines together with their receptors are constitutively expressed in different brain regions and function in neural development (Bajetto, et al., 2001, Tran, et al., 2007, Tran, et al., 2004). Certain chemokine receptors, particularly the CXC chemokine receptor 4 (CXCR4), are found in proliferative regions of the developing central nervous system in neurons and neuronal precursors, and SDF-1, the ligand for CXCR4, and other chemokines have been observed to be expressed in nearby astrocytes, microglia, and neurons, particularly after brain injury or infection (Bajetto, et al., 2001, Li and Ransohoff, 2008, Lu, et al., 2002). Chemokine signaling mediated through the CXCR4 receptor is of central importance in guiding the migration of populations of neural progenitors during development (Ma, et al., 1998, Zou, et al., 1998). Moreover, chemokines modulate synaptic activity during neurogenesis in adults: SDF-1 enhances GABAergic transmission to neural progenitors in the murine DG (Bhattacharyya, et al., 2008). These observations raise the question as to the role of CXCR4 signaling in the brain's response to ischemia: it is likely that CXCR4 participates in regulating the migration of neural progenitors to the periphery of the ischemic lesion (Ohab and Carmichael, 2008). It is not known, however, whether CXCR4 signaling also plays a role in enhancing synaptic inputs to developing neurons once they have arrived in the ischemic lesion borderzone. Hence, in this study, we evaluated the effect of SDF-1 on the electrophysiological properties, including GABAergic signaling, of immature neural cells stimulated by cerebral ischemia-reperfusion injury of the striatum and cortex in mice.

MATERIALS AND METHODS

Procedures performed on mice were approved by the Northwestern University Institutional Animal Care and Use Committee and were in accordance with NIH guidelines for the use of animals in research.

Transgenic mice

DCX-enhanced green fluorescent protein (EGFP) transgenic mice and SDF-1-monomeric red fluorescent protein (mRFP)/CXCR4-EGFP bi-transgenic mice used in this study have been described by us previously (Bhattacharyya, et al., 2008).

Experimental cerebral ischemia

Transient MCAO with an intra-luminal suture was used as the model of cerebral ischemia-reperfusion in mice. For the electrophysiology endpoint (due to the difficulty involved in patch clamping cells in older animals), male DCX-EGFP mice, 4 to 5 weeks old, were used. Mice were anesthetized with isoflurane, 5% for induction, 1.5% for maintenance in an O₂ (0.05 L/min) –room air (1 L/min) carrier; positioned supine under the dissecting microscope; and sterilely prepped and draped. During the MCAO procedure, rectal temperature was monitored and maintained between 36.5 and 37.5°C using a homoeothermic blanket-probe system (Harvard Apparatus, Holliston, MA). The neck was opened in the midline, and the salivary glands were bluntly dissected allowing access to the right common carotid artery (CCA). The CCA was freed from the surrounding tissues and tied off. The external carotid artery (ECA) was divided and the internal carotid artery (ICA) take-off was dissected free of surrounding tissue. A temporary clip was applied to the ICA and the CCA was cannulated with a 7–0 nylon filament with a resin-coated tip. Perfusion of the right MCA was monitored with laser Doppler flowmetry (Perimed Instruments, Inc., Philadelphia, PA) with the tip of the probe placed at the temporal bone through a small incision in the skin and temporalis muscle. The ICA clip was removed, and the filament was advanced 10–11 mm from the ICA origin until MCA flow dropped. The filament was secured in place and the neck incision sutured. The animal was then awakened for the 75-minute duration of the occlusion. Neurologic score (0 - no deficit; 1.0 - body twisting when suspended; 2.0 - circling; 3.0 - rolling; 4.0 - no movement) was obtained 30 minutes into the occlusion. A score of 1.5 was used when the animal was veering, but not circling, to the right when walking. After 75 minutes of occlusion, the animal was reanesthetized, and the occluding filament was removed from the ICA (with real time laser Doppler flowmetry in order to document reperfusion). The neck incision and the temporal skin incision were then sutured. Animals were survived for up to four weeks after cerebral ischemia-reperfusion.

For the confocal imaging endpoint, male DCX-EGFP or SDF-1-mRFP/CXCR4-EGFP mice, 8 to 12 weeks old, were used. The surgical protocol used for the older mice differed from the one used for the younger mice due to differences in technical success observed in the pilot phase: instead of 75 minutes of ischemia with intra-ischemic awakening, older mice were occluded for 30 minutes while continuously under anesthesia. Post-ischemic neurological scores were recorded 60 minutes after reperfusion, and the animals were survived for up to four weeks.

The rate of intraoperative mortality/technical failure was 9%, and mortality during the subsequent month in mice that survived surgery was 10% for DCX-EGFP mice and 30% for SDF-1-mRFP/CXCR4-EGFP bitransgenic mice.

Lentivirus injection

Five – six week old DCX-EGFP mice were anesthetized with an intraperitoneal injection of Avertin (33 mg/kg; Sigma-Aldrich Corp., St. Louis, MO) and placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IL). mCherry-expressing lentivirus (2 μ l; GeneCopoeia, Rockville, MD) was injected into the SVZ at the anterior limb of the right lateral ventricle at the following coordinates: 1.94 mm posterior to bregma, 2.8 mm lateral to the midline; and 2.4–2.8 mm ventral to the pia. Seven days later, mice were subjected to MCAO, and were euthanized four weeks later. Brains were sectioned on a vibratome and visualized using confocal microscopy as described below.

Electrophysiology

Brain slices were prepared from DCX-EGFP mice three to four weeks after cerebral ischemia-reperfusion. For each experiment, coronal sections of cortical region (300 μ m) were obtained with a vibratome and kept for 30 min at 37°C in oxygenated standard artificial CSF (ACSF) (in mM): 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 1.5 CaCl₂, 1 MgSO₄, and 10 glucose, saturated with 95% O₂/5% CO₂ at pH 7.4. Slices were stored in a modified interface chamber for 30–40 min at 37°C and then maintained at room temperature until being transferred to the recording chamber in oxygenated standard ACSF containing (in mM) 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 1.5 CaCl₂, 1 MgSO₄, and 10 glucose, saturated with 95% O₂ and 5% CO₂ at pH 7.4.

Whole-cell patch-clamp recordings were performed from EGFP-positive cells located in the striatal (medial) borderzone of the lesion (identified grossly by visual inspection) and 10–20 μ m below the surface of the slice. Patch pipettes were filled with a solution that mimicked the intracellular environment and that contained (in mM) 150 KCl, 10 HEPES, 4 Mg₂ATP, 0.5 NaGTP, and 10 phosphocreatine. The pH was adjusted to 7.3 with KOH. The input resistance (IR) and resting membrane potential were measured from each cell. SDF-1 (40 nM; BD Biosciences, Chicago, IL), AMD 3100 (Sigma), bicuculline methiodide (BIC; Sigma), 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX; Sigma), dl-2-amino-phosphonovaleric acid (DAP5, Tocris Bioscience, Minneapolis, MN), tetrodotoxin (TTX; Tocris), and CdCl₂ were applied by either focal or bath application. Cells were clamped at –70 mV, and recordings were obtained in presence of 10 μ M CNQX and 50 μ M DAP5.

Brain tissue preparation and immunolabeling

Mice were euthanized 7, 10, 15, 21, or 30 days after cerebral ischemia-reperfusion and perfused transcardially with phosphate-buffered saline (PBS), followed by a freshly prepared solution of 4% paraformaldehyde (PFA) in PBS, pH 7.4. The brains were rapidly removed and postfixed for 48h in 4% PFA at 4°C. Forty-micrometer coronal sections were cut with a vibratome and collected in cold PBS. Free-floating sections were either analyzed directly by confocal microscopy to observe for epifluorescence (see below) or prepared for immunolabeling.

For immunolabeling, sections containing the ischemic lesion were pre-treated for 90 minutes at 25°C in PBS containing 0.1% Triton X-100 and 4% normal goat serum supplemented with 4% bovine serum albumin. The sections were then incubated overnight at 4°C with primary antibodies. To determine the phenotypes of perilesional cells, the following primary antibodies were used: 1) Iba-1 (rabbit IgG, 1:300 dilution; Wako Chemicals USA, Richmond, VA) for microglia; 2) F4/80 (rat IgG2b, k, 1:200 dilution; Invitrogen, Camarillo, CA) for macrophages; 3) DCX (mouse IgG1, 1:200 dilution; Millipore, Temecula, CA) for migratory neuroblasts; 4) glial fibrillary acidic protein (GFAP; mouse monoclonal, 1:300 dilution; Sigma) for astrocytes; and 5) von Willebrand factor (vWF; rabbit polyclonal, 1:100 dilution; Millipore) for blood vessels.

After incubation with primary antibodies, sections were washed with PBS five times, 10 minutes each, and then incubated for 90 minutes in Cy5 (Jackson ImmunoResearch, West Grove, PA) or Alexa-633 (Molecular Probes, Eugene, OR) conjugated secondary antibodies. Sections were finally washed and mounted in Vectashield (Vector Laboratories, Burlingame, CA).

Confocal microscopy

Images were captured using a confocal microscope (FV10i, Olympus Corporation of America, Center Valley, PA) and analyzed using Fluoview acquisition software (Version 02.01c; Olympus). All captured images were exported to Adobe Photoshop CS3 (Adobe, San Jose, CA), and adjustments were made to the brightness and contrast to reflect true colors as closely as possible.

Statistical analysis

Statistical analysis was performed using Sigma Stat 3.0 (SPSS, Chicago, IL). Mean $\pm$ SD or median (25% - 75% quartile) is presented unless otherwise indicated.

RESULTS

Animals

Weights on the day of transient MCAO and ischemic parameters are presented in Table 1. Overall mortality was 10% for DCX-EGFP mice and 30% for SDF-1-mRFP/CXCR4-EGFP bitransgenic mice).

Perilesional DCX-expressing cells

In normal animals, DCX-EGFP cells were observed in the SVZ of the lateral ventricle (data not shown). After cerebral ischemia-reperfusion, a high level of fluorescence was present along the lateral ventricle in the ipsilateral hemisphere resulting from hypertrophy of the SVZ (Fig. 1 A, B). Groups of DCX-EGFP cells were also located between the SVZ and the infarct (Fig. 1 B – D). The perilesional DCX-EGFP cells' location, as well as their bipolar, elongated morphology suggested that they were migratory neuroblasts which originated in the SVZ and were migrating under the corpus callosum and through more ventral striatal parenchyma towards the infarct. To demonstrate that the DCX-EGFP cells were functional as immature neurons, we performed electrophysiological recordings on cells specifically localized at the edge of the infarct as well as in the SVZ (Fig. 1 F–G). TTX-sensitive voltage-dependent Na currents were present in DCX-EGFP cells at the infarct borderzone, confirming their neuronal phenotype (Fig. 1 E), whereas no currents were detected in DCX-EGFP cells localized in the SVZ (Fig. 1 F).

In order to confirm that the SVZ was indeed the origin of the DCX-EGFP cells we injected an mCherry-expressing lentivirus into the ipsilateral lateral ventricle one week prior to experimental stroke. Four weeks after injury, yellow perilesional DCX-EGFP co-expressing the mCherry signal were observed (Fig. 2), suggesting that they originated in the SVZ.

We then evaluated GABA signaling in DCX-EGFP cells three to four weeks after ischemia-reperfusion injury. Because immature neurons have high intracellular chloride ion concentrations, GABA-induced tonic currents are excitatory (inward). Application of GABA (200 μ M) to brain slices in our experiments resulted in inward currents in perilesional DCX-EGFP cells (Fig. 3 A). Recordings from these cells demonstrated spontaneous post synaptic currents (PSCs) which were blocked by the application of the GABA-A receptor antagonist, BIC, (Fig. 3 B). The application of BIC also produced an outward current, indicating the presence of both tonic and phasic components of GABAergic transmission at these

synapses, as also previously observed when recording from neural stem cells in the DG (Bhattacharyya, et al., 2008).

We have previously demonstrated that during maturation of neural progenitors in the adult DG, the chemokine SDF-1 acts as a cotransmitter with GABA and enhances GABA_A-mediated inputs to developing progenitors (Bhattacharyya, et al., 2008). As the expression of SDF-1 is strongly upregulated in the ischemic lesion (see below), we also wondered if SDF-1 would enhance GABAergic inputs to developing progenitors in the context of post-ischemic repair. Under whole cell voltage clamp conditions application of SDF-1 to the perilesional DCX-EGFP cells (Fig. 3 C) resulted in an increased frequency (Fig. 3 D) and amplitude (Fig. 3 E) of GABA-mediated PSCs together with the development of an inward current. These effects of SDF-1 could be reversed by BIC (Fig. 3 C – E) indicating that, as in the DG, they were the result of enhancement of GABA_A signaling. When SDF-1 was applied, it regularly produced a long-lasting inward current (Fig. 3 F, G). The SDF-1-induced inward current was completely reversed not only by BIC (Fig. 3 F, H) but also by the CXCR4 antagonist, AMD 3100, (Fig. 3 G, H) which suggests that the effects of SDF-1 were mediated by activation of CXCR4 receptors. These electrophysiological data imply that initial inputs to immature perilesional neurons are GABAergic in nature and, as in the DG, are modulated by SDF-1.

Perilesional CXCR4-expressing cells

Bitransgenic SDF-1-mRFP/CXCR4-EGFP mice (Bhattacharyya, et al., 2008) were utilized to evaluate the phenotype of the CXCR4-EGFP cells and expression of SDF-1-mRFP. As we have previously reported (Tran, et al., 2007), the major site of expression of CXCR4 in the postnatal mouse brain is normally the SVZ and DG. After cerebral ischemia-reperfusion, the picture was changed: increased numbers of CXCR4-EGFP cells were observed for up to three weeks after injury in the SVZ and in the ischemic lesions, but not in contralateral striatal and cortical regions (Fig. 4 A, B). CXCR4-EGFP cells did not express astrocytic or neuronal markers (data not shown); however, many CXCR4-EGFP cells were labeled with IBA-1 indicating they were activated microglia (Fig. 4 C – H), and many were positive for F4/80, further corroborating their identity as macrophages (Fig. 5 A – C). Lesional immune cell infiltration was qualitatively greater in animals surviving for seven or fifteen days than in those surviving for three weeks (Fig. 4 A, B). Based on the electrophysiological results, we expected CXCR4-EGFP cells to express DCX, but no such cells were discernible either in the SVZ or perilesionally at any of the survival endpoints evaluated.

The localization of SDF-1 was assessed using mice containing an SDF-1-mRFP fusion protein transgene. SDF-1-mRFP was enriched in ischemic lesions and appeared punctate (Fig. 5 D – I) in keeping with our previous observation that SDF-1 is stored in secretory vesicles (Bhattacharyya, et al., 2008). SDF-1-mRFP puncta were observed along vascular structures (Fig. 5 E, F), and SDF-1-mRFP was also found to be coincident with IBA-1-positive cells (Fig. 5 G – I). As with CXCR4, there was little SDF-1-mRFP expression in the contralateral hemisphere (Fig. 4 A, B).

DISCUSSION

There is a paucity of acute treatments for ischemic stroke, and hundreds of thousands of patients throughout the world are disabled each year. Physical rehabilitation is the main strategy aimed at improving function after injury, but the past several decades of research on the neurobiology of brain injury led to the idea that the natural reparative processes of the brain could be pharmacologically enhanced to improve functional outcome. One of the key observations was that the adult brain is capable of neurogenesis under physiologic and pathologic conditions (Lindvall and Kokaia, 2011). In hemispheric ischemic stroke, the SVZ

serves as a pool of stem cells and progenitors which can migrate toward the lesion borderzone, which is a region of robust post-injury plasticity (Ohab and Carmichael, 2008, Zhang, et al., 2008). In the infarct borderzone, the progenitors are thought to develop into neurons and integrate into neuronal circuits. However, little is known about what modulates this process, how durable it is, and how it contributes to ultimate functional improvement.

It is also well known that injury due to ischemia-reperfusion results in an inflammatory response in the brain, and a major component of this response is the expression of inflammatory cytokines and related molecules (Lakhan, et al., 2009). In the context of the present discussion, the effects of such molecules on injury related neurogenesis are of particular interest. Neural progenitor cells express receptors for chemokines such as CXCR4, the receptor for SDF-1 (Tran et al 2004, 2007), and SDF-1 is responsible for guiding the migration of stem cells during formation of structures during embryogenesis, including the hippocampal DG (Lu, et al., 2002). In addition, SDF-1 is expressed in the adult DG stem cell niche and cooperates with GABAergic signaling in synaptic transmission to developing granule cells (Bhattacharyya, et al., 2008). It has also been demonstrated that SDF-1 functions as a chemoattractant for neural progenitors migrating to injured brain regions during repair (Ohab and Carmichael, 2008).

In the current study, we initially focused on SVZ neural progenitor cells which express DCX. Previous electrophysiological characterization in adult mice showed that DCX-expressing cells constitute a heterogeneous population: some cells exhibit no neuronal characteristics; some show features consistent with immature neurons; and some are mature neurons (Walker, et al., 2007). Liu et al. had previously evaluated DCX-expressing SVZ cells ipsilateral to ischemic lesions and found that the resting membrane potentials of ipsilateral DCX-expressing cells were hyperpolarized compared to DCX-expressing cells in the contralateral SVZ, and that ipsilaterally located cells expressed TTX-sensitive Na currents (Liu, et al., 2009). In the current study, we evaluated the electrophysiological properties of DCX-expressing cells up to four weeks after injury: in the SVZ these cells did not exhibit Na currents; while in the vicinity of ischemic lesions they exhibited robust TTX and voltage-sensitive Na currents suggesting that they were immature neurons which migrated from the SVZ. We further confirmed the origin of the DCX-EGFP using intraventricular/SVZ lentivirus injections.

DCX-EGFP cells in the infarct borderzone exhibited GABAergic PSCs characteristic of immature neurons. GABAergic activity depolarizes immature DG neural progenitors and increases intracellular calcium resulting in further maturation (Ge, et al., 2006, Tozuka, et al., 2005). Similar to GABAergic inputs to immature granule cells during adult hippocampal DG neurogenesis (Bhattacharyya, et al., 2008), GABAergic inputs to perilesional DCX-cells were enhanced by SDF-1 in a CXCR4-specific fashion, suggesting that SDF-1 may also play a role in development of immature neurons during the post-ischemic repair process.

Like DCX, CXCR4 is highly expressed by neuronal progenitors in the adult SVZ (Tran, et al., 2007), and so it would be reasonable to suppose that migrating DCX-expressing cells also express CXCR4 receptors. Nevertheless, we were unable to demonstrate DCX and CXCR4 co-localization in cells, perhaps owing to the fact that the majority of CXCR4-expressing cells in proximity to the area of damage were microglia or macrophages, which presumably greatly outnumbered migrating neuroblasts. On the other hand, recording from DCX-EGFP cells in the same area is not subject to the same problem, and the effects of SDF-1 on GABAergic inputs to DCX-expressing neuroblasts were clearly demonstrated. However, given the observation that many microglia and macrophages express CXCR4 following ischemia, it is also possible that SDF-1-induced release of mediators from these

cells which then may have played a role in the observed electrophysiological responses in DCX-expressing cells.

The potential role of CXCR4 signaling in enhancing GABAergic inputs to neurons in the infarct borderzone is consistent with the overall role of SDF-1 in the repair process after ischemia-reperfusion. As we have also demonstrated here, SDF-1 expression was associated with endothelial and microglial cells, and SDF-1 release from either source could be important in influencing synaptic inputs to new neurons. The observation of SDF-1 in endothelial cells is also consistent with the role of this chemokine in the neovascularization response to brain injury (Ohab, et al., 2006).

The ultimate effect of SDF-1/CXCR4-mediated modulation of GABAergic transmission on the development of newly generated neurons, plasticity, and functional recovery after cerebral ischemia-reperfusion is unknown and, therefore, the potential clinical utility of modulating the chemokine axis during repair after ischemic stroke is unclear at this time. However, the role of GABAergic transmission after ischemic injury has been recently evaluated in mice, albeit in a model of permanent ischemia with cortical lesional involvement only (Clarkson, et al., 2010). In that study, perilesional neurons were tonically inhibited through extrasynaptic GABA_A receptors, and pharmacologic attenuation of GABAergic neurotransmission after injury resulted in improved motor recovery during long-term survival (Clarkson, et al., 2010). The model used in our study is, however, different: it features injury due to ischemia and reperfusion; lesions involve both striatum and cortex; and the focus is on immature neurons. Thus, inhibiting GABAergic or chemokine signaling after cerebral ischemic injury may have either beneficial or detrimental effects on striatal immature neurons, and it is difficult to predict without further studies what the ultimate functional outcome is in our model.

Although the experiments reported here illustrate the potential role of SDF-1 in the establishment of synaptic inputs to new neurons in the context of brain repair, it is likely that other cytokines also participate in these events. As demonstrated by our laboratory and others, neural progenitors in the SVZ express a variety of cytokine/chemokine receptors that would allow them to respond to several important cytokines that are expressed in the context of brain damage and infection (Tran, et al., 2007). Like SDF-1, the chemokine MCP-1/CCL2 also participates in the migratory response of neural progenitors in the brain (Belmadani, et al., 2006): CCR2 receptors are expressed by migrating neural progenitors; and MCP-1 can enhance GABA inputs to these cells (our unpublished observations). Thus, a complete understanding of the role of the innate immune response on neural progenitor development must involve consideration of these other molecules as well.

Acknowledgments

NIH # R01DA013141 (PI: Richard J Miller)

ABBREVIATIONS

ACSF	artificial CSF
BIC	bicuculline methiodide
CCA	common carotid artery
CXCR4	CXC chemokine receptor 4
DCX	doublecortin

DG	dentate gyrus
ECA	external carotid artery
EGFP	enhanced green fluorescent protein
GABA	-aminobutyric acid
GFAP	glial fibrillary acidic protein
ICA	internal carotid artery
MCA	middle cerebral artery
MCAO	middle cerebral artery occlusion
mRFP	monomeric red fluorescent protein
PBS	phosphate buffered saline
PFA	paraformaldehyde
SDF-1	stromal derived factor-1 (CXCL12)
SGZ	subgranular zone
SVZ	subventricular zone
TTX	tetrodotoxin
vWF	von Willebrand factor

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Figure 1.

Doublecortin (DCX)-EGFP-positive immature neurons were found in lesional tissue up to four weeks after transient cerebral ischemia. (A) Low-power confocal image of a coronal section of mouse brain showing approximate locations of the subventricular zone (SVZ) region near the ischemic lesion portrayed in panel B and peri-lesional tissue regions portrayed in panels C and D. Scale bar = 500 μ m; boxes not to scale. (B) The SVZ ipsilateral to the lesion contained DCX-EGFP cells (arrowhead). Groups of DCX-EGFP cells (arrow) were also located between the SVZ and the lesion, which is to the right, outside of the image. * = lateral ventricle; scale bar = 250 μ m. (C, D) Higher-power images of DCX-EGFP cell bodies and processes in the peri-lesional region, some exhibiting a bipolar morphology. cc = corpus callosum; scale bar = 7 μ m. (E) Peri-lesional DCX-EGFP cells exhibited voltage-dependent sodium currents recorded under voltage clamp conditions (RMP -61 ± 2 mV with 1.2 ± 1 g resistance). Inward currents were blocked with tetrodotoxin (TTX), 500nM, $n = 9$ cells. (F) Electrophysiological recording from DCX-EGFP cells in the SVZ (non-migratory; arrowhead in panel B) showed no voltage-dependent currents under voltage clamp conditions, $n = 9$ out of 9 cells (100%), with RMP value of -68 ± 2.1 mV and 5.1 ± 1 (g) resistance. (G) Illustration of the voltage steps applied to record voltage dependent currents from SVZ and perilesional cells.

Figure 2.

Doublecortin (DCX)-EGFP-positive immature neurons in lesional tissue four weeks after experimental stroke originated in the subventricular zone (SVZ). (A) Low-power coronal view of the contralateral (non-ischemic) striatum combining the red (m Cherry), green (EGFP), and blue (Hoechst) channels. cc = corpus callosum; ac = anterior commissure; the midline is to the right of the SVZ, which is indicated with the arrow. (B) Low-power coronal view of the ipsilateral (ischemic) striatum showing the lesion (area demarcated with the white line). cc = corpus callosum; ac = anterior commissure; the midline is to the left of the SVZ, which is indicated with the arrow. Scale bar (A, B) = 250 μ m. (C) Two CXCR4-EGFP cells in lesional tissue, one of which (arrowhead) is also labeled with lentivirus encoding mCherry (D, E) which had been injected into the SVZ at the lateral ventricle prior to experimental stroke. Magnification = 120X; scale bar = 10 μ m. In contrast to DCX-EGFP expressing cells, cells co-expressing lentivirus did not appear healthy.

Figure 3.

The chemokine stromal-derived growth factor-1 (SDF-1, 40 nM) modulated GABAergic transmission from perilesional doublecortin (DCX)-EGFP-positive immature neurons three to four weeks after transient cerebral ischemia. Electrophysiological recordings were made in DCX-EGFP-expressing cells in acutely isolated cortical slices. (A) GABA, 200 μ M, produced inward currents, $n = 4$ out of 6 cells (67% responders), and (B) bicuculline (BIC; GABA_A receptor antagonist), 50 nM, produced outward currents, $n = 8$ out of 10 cells (80% responders), in peri-lesional DCX-EGFP cells. (C) Spontaneous post-synaptic currents (PSCs) were recorded from peri-lesional DCX-EGFP cells. (D) PSC frequencies were significantly ($p < 0.001$) enhanced by SDF-1, 40 nM, and attenuated by BIC, 50 nM, $n = 7$ out of 10 cells (70% responders), error bars = SEM. (E) PSC amplitudes were significantly ($p < 0.001$) increased in presence SDF-1 and significantly decreased ($p < 0.001$) in presence of BIC, $n = 7$ out of 10 cells (70% responders), error bars = SEM. SDF-1 produced GABAergic tonic inward current in peri-ischemic DCX-EGFP positive cells (F–H) and was antagonized by BIC (F and H), $n = 6$ out of 10 cells (60% responders), and AMD 3100 (G, H), $n = 4$ out of 5 cells (80% responders), error bars = SEM.

Figure 4.

CXCR4-EGFP cells and SDF-1-mRFP co-localized in ischemic lesions 7 and 21 days after experimental stroke in CXCR4-EGFP/SDF-1-mRFP bi-transgenic mice. Low-power images of brain sections from CXCR4-EGFP/SDF-1-mRFP bi-transgenic mice surviving for 7 (A) or 21 (B) days after cerebral ischemia-reperfusion showing lesional (asterisk) CXCR4-EGFP (green) and SDF-1-mRFP (red) signals. In (B), the arrows indicate CXCR4-EGFP signal in the subventricular zone (SVZ) on the contralateral and ipsilateral side. CXCR4-EGFP cells expressed IBA1 (arrows) in CXCR4-EGFP/SDF-1-mRFP bi-transgenic mice 7 (C – E) and 21 (F – H) days after experimental stroke. CXCR4-EGFP (green), IBA1 (red); scale bar (C – H) = 20 μm.

Figure 5.

The macrophage marker, F4/80, was associated with CXCR4-EGFP cells three weeks after experimental stroke. (A) A perilesional CXCR4-EGFP cell (arrowhead); (B) F4/80 immunolabeled cell (arrowhead); and (C) combined red-green image showing association of F4/80 labeling with CXCR4-EGFP cells (arrowhead). Scale bar = 10 μm. (D) CXCR4-expressing cells were in close association with SDF-mRFP puncta in SDF-1-mRFP/CXCR4-EGFP bi-transgenic mice three weeks after transient cerebral ischemia. Scale bar = 10 μm. (E, F) SDF-1-mRFP (red) was associated with von Willebrand Factor (vWF)-immunoreactive blood vessels (green; arrowheads). Scale bar = 5 μm. (G - I) SDF-1-mRFP puncta were associated with IBA-1-immunoreactive microglial cells (crosshairs). Scale bar = 2.5 μm.

Table 1

	Weight, g	Rectal temperature, °C	Occlusion, % baseline	Postoperative neurologic score
Electrophysiology DCX-EGFP (n = 19)	19.1 ± 2.7	36.6 ± 0.3	21 ± 11	1.0 (1.0–2.0)
Confocal Imaging DCX-EGFP (n = 14) ^a	22.4 ± 3.2	37.0 ± 0.1	15 ± 4	2.0 (1.0–2.0)
DCX-EGFP/lentivirus- mCherry (n = 6)	24.0 ± 2.0	36.8 ± 0.2	17 ± 8	1.5 (1.0–2.0)
SDF1-mRFP1/CXCR4-EGFP (n = 13) ^b	27.3 ± 0.6	37.1 ± 0.2	18 ± 15	2.0 (1.0–2.0)

^aThree animals at 7 days of survival; two at 10 days; four at 15 days; and five at 21 days.

^bFive animals at 7 and 15 days of survival; three animals at 21 days of survival.